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INDUCED POLYPLOIDY IN *THEOBROMA CACAO* L. AND RELATED SPECIES

By RONALD KNIGHT*
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Theobroma cacao L. is a tropical fruit tree with a diploid complement of twenty chromosomes. No instances of naturally occurring polyploidy have been recorded for this species. Posnette (1943) attempted to induce polyploidy in seedlings by using 0·25 per cent.† colchicine, but although he states that these seedlings showed morphological features usually associated with polyploidy, no confirmation by cytological methods was attempted.

Great difficulty has been experienced in the past in the production of hybrids between *T. cacao* and related species of *Theobroma* which are known to have twenty chromosomes. With some species cross-fertilization is apparently effected but the embryo fails to develop, while with others a good bean is formed but the seedling fails to grow beyond the three- or four-leaf stage. The investigation described in this paper was a preliminary to attempting the production of tetraploid hybrids in two ways: one by induction of polyploidy in the parental species before crossing, and the other by early treatment of diploid hybrids.

As *T. cacao* takes four or five years to come into bearing and some of the other species still longer, this study deals only with the induction of polyploidy and its effect on the vegetative growth of the species.

Materials and methods.

Several types of *T. cacao* are grown in the Gold Coast. These are introductions from South America and are usually named after population groups occurring there. (Knight and Rogers, 1955). Representatives of these types have been used in the experiments to induce polyploidy. Seedlings of the Amelonado type were the first to be treated as it was thought that a comparison between the most generally grown type of cacao and its tetraploid form would be most desirable. Hybrids between the Upper Amazon, Amelonado and Trinitario types have also been treated in the hope that they might give tetraploids more fertile than those of the parental groups. The four related species that were treated were *T. grandiflora* K. Schum., *T. microcarpa* Mart., *T. speciosa* Spreng. and *T. bicolor* Humb. et Bonpl.

The drug colchicine was applied at several different concentrations and at various stages in the life of the plant. In the first experiment a similar technique to that of Hunter (1954) was used. Colchicine, incorporated in 0·8 per cent. agar, was poured into celluloid-coated gelatine capsules which were then forced over the apical bud of the plant. It was found that a suitable stage at which to carry out this application was soon after germination when the cotyledons had opened and the shoot was visible. In making the application at this stage both the apical shoot and the axillary buds were treated. *T. microcarpa* and *grandiflora* are hypogeal, so that with these species the axillary buds were not directly in contact with the colchicine agar. Apical buds

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† Misprinted in the original as 25 per cent.

of seedlings twenty months old and the buds of rooted cuttings were treated by the same method as that used for young seedlings.

In a different method of application the cacao beans were allowed to germinate until the radicle had reached a length of two to three centimetres. The beans were then placed vertically with the radicles pushed into agar incorporating the colchicine. After treatment, they were placed in test tubes and left until secondary roots had developed before planting out in baskets.

A range of from 0·1 to 1·5 per cent. colchicine was used. It was soon found that the agar dried out if the treatment lasted longer than twenty-four hours. Treatment for approximately twenty-four hours was adopted as the standard procedure. The results obtained with these techniques, especially when the colchicine was applied to the apical shoot, were fairly satisfactory and further modification was not attempted.

Acetic lacmoid squashes of rapidly growing flush leaves were made to determine the chromosome number. After fixation the leaves were macerated for 5 minutes in equal parts of HCl and 95 per cent. alcohol, hardened in Carnoy for 10 minutes and then stained. The chromosomes were readily counted by this technique and it was soon established that tetraploid forms of cacao had been produced. When the distribution of diploid and tetraploid cells in a leaf had to be determined, sections were cut and stained in crystal violet. Detailed cytological investigation of the apical and axillary growing points would be necessary to establish with certainty that all the tissues of a treated shoot were tetraploid. This was not attempted. A treated plant that showed no diploid cells in leaf squashes or in sections of the leaves was considered to have at least two of its histogenic layers polyploid. Fig. 1 illustrates a tetraploid epidermal cell.

RESULTS.

A colchicine concentration of 0·6 per cent. in agar was found to be optimal for the induction of polyploidy in the shoot. Of 18 plants treated at this concentration in one experiment, 14 were found to have at least the epidermis tetraploid. At concentrations above 1·0 per cent. there was an ever-increasing number of deaths due to the toxicity of the colchicine. At concentrations lower than 0·6 per cent. there was a tendency for the plants to grow away from the tetraploid state, although a few plants with all their leaves tetraploid were produced at a concentration of 0·1 per cent.

It was found that colchicine is translocated in the shoot of *T. cacao* and the related species, buds distant from those treated growing out as tetraploid shoots. In one plant of the hypogeal *T. microcarpa* the treated shoot died and was replaced by two axillary shoots, one of which had a tetraploid epidermis. In this case the colchicine was translocated a distance of 4 cm. down the shoot.

In view of the translocation of the colchicine it was hoped that treatment of either root or shoot would result in the induction of polyploidy in both. When cacao roots were treated with 0·6 per cent. colchicine there were many deaths. Of 109 plants treated in this way only 20 were alive three months later but 15 of these had developed shoots with tetraploid cells. On examining the roots of 20 plants, the shoots of which had been successfully treated, not one was found to have tetraploid cells. It is evident that the colchicine is not translocated to the root tip in sufficient concentration to induce polyploidy, or that the roots in subsequent growth revert to

the diploid state. For the production of plants with tetraploid roots and shoots, the roots may be treated or cuttings may be taken from tetraploid shoots.

CHARACTERS ASSOCIATED WITH POLYPLOIDY.

Characters that would enable a quick determination of polyploidy were sought. Counts of stomata per unit area and stomatal size measurements were made on mature leaves by taking cork-borer punches from the mature leaf lamina and mounting in glycerine before examination under the microscope. The leaf was not severely affected by this operation. Where the epidermis was tetraploid the average length of the guard cells was $18 \cdot 28\mu \pm 0 \cdot 15$, in contrast to $13 \cdot 62\mu \pm 0 \cdot 15$, for the diploids. Stomatal frequency was more subject to environmental variation than stomatal size. A leaf developing in an exposed position had a greater number of stomata per unit area than one in a sheltered position. It was also found that the stomatal frequency was less on the first-formed leaves of a seedling than on leaves developing later. Stomatal frequency was, therefore, used to diagnose a tetraploid epidermis only after comparison with a control leaf developing under similar conditions. When the stomatal frequency of leaves growing under comparable conditions was determined it was found that there were on an average 35 per cent. fewer stomata per unit area of a tetraploid epidermis than of a diploid epidermis.

Ploidy in the leaf results in changes of size and shape. Twenty-one plants of Amelonado cacao were analysed for changes in leaf shape and thickness. Of these, seven were tetraploid in both epidermis and mesophyll, seven were periclinal chimeras with a tetraploid mesophyll and diploid epidermis and seven were completely diploid. The normal cacao leaf is oblanceolate whereas in tetraploid plants the leaves are more obovate. A measure of this change in shape can be obtained by dividing the distance of the broadest part of the leaf from the peduncle by the leaf length and converting to a percentage. A higher percentage is obtained with an obovate leaf. The percentage was calculated for 5 leaves of each tree and an average taken. At the same time, leaf thickness was measured with a micrometer for the 5 leaves and this also was averaged. The results are given in Table I. It is evident that a tetraploid mesophyll results in a differently shaped and thicker leaf.

Two plants were found in which the mesophyll was diploid and the epidermis tetraploid. These more closely resembled diploid plants, having a leaf-shape percentage of $52 \cdot 5$ and $52 \cdot 9$ and a leaf thickness of $6 \cdot 10$ and $6 \cdot 34$.

CHIMERAS.

The first formed leaves after treatment with colchicine showed a complicated mosaic. Certain areas were thicker and of a darker green and they bore longer stomata than the remainder of the leaf. This complicated mosaic probably arose through the doubling of the chromosome complement in cells which were in a state of active division at the time of treatment, whereas the remainder of the leaf developed from cells unaffected by the colchicine and in the normal diploid state. Leaves that developed complicated mosaics evidently had cells in a state of division at the time of treatment. Subsequent leaves might be sectorial, mericlinal or periclinal chimeras. The sectoring of leaves formed some time after treatment nearly always involved half of the leaf. Fig. 2 is a photograph of two sectored leaves in which the broader side is

tetraploid and the narrower side diploid. These leaves also illustrate the change in shape, the darker colour and the more pronounced veins of the tetraploid halves.

TABLE I.

Analysis of leaf shape and thickness.

Diploid		Chimera		Tetraploid	
Leaf shape %	Mean thickness (0·001 in.)	Leaf shape %	Mean thickness (0·001 in.)	Leaf shape %	Mean thickness (0·001 in.)
52·7	6·0	54·6	7·8	57·0	7·6
51·1	5·6	55·8	9·4	59·7	6·3
52·5	7·1	56·9	6·8	58·3	6·9
50·9	6·7	58·6	6·8	56·8	8·1
50·9	6·6	55·8	5·8	57·6	7·8
53·5	5·9	59·1	9·1	57·4	8·5
55·1	6·2	55·9	6·2	60·1	8·1
Means:					
52·39	*6·30*	*56·67*	*7·41*	*58·13*	*7·61*

Leaf Shape:
L.S.D. between means for P = 0·05, 0·01 and 0·001 are 1·63, 2·23 and 3·04 respectively. Chimeras and tetraploids are significantly different at P = 0·01 from diploids.

Leaf thickness:
L.S.D. between means for P = 0·05, 0·01 and 0·001 are 1·09, 1·49 and 2·03 respectively. Chimeras and tetraploids are significantly different at P = 0·05 from diploids.

Recognition of periclinal chimeras, as will be discussed later, is of considerable importance. Marks (1953) found that the Double Williams' pear was a periclinal chimera with a diploid epidermis and all other tissues tetraploid. The stomatal frequency per unit area of Double Williams' was compared with that of Williams' and found to be significantly less. He attributed the difference to the "stretching" of the diploid epidermis by the tetraploid mesophyll. It was hoped that if this phenomenon occurred in cacao it might be used to distinguish periclinal chimeras.

Leaves that are mericlinal chimeras are particularly suitable for testing the occurrence of this phenomenon. Any environmental effect on stomatal frequency, colour of the leaf and vein size should be largely overcome by the use of halves of the same leaf. A study of stomatal frequency was made on ten sectored leaves in which it was evident that one half had a tetraploid and the other a diploid mesophyll. These leaves with a sectored mesophyll can have either a completely tetraploid or a completely diploid epidermis. Five leaves were chosen with a diploid and five with a tetraploid epidermis. Twenty-five counts were made on each half leaf, making a total of five hundred readings for the ten leaves. The results were statistically analysed for differences in stomatal frequency in the same type of epidermis overlying the two different types of mesophyll. With the diploid epidermis overlying tetraploid and diploid mesophylls the means of one hundred and twenty-five readings were respectively $14{\cdot}1 \pm 0{\cdot}19$ and $13{\cdot}7 \pm 0{\cdot}19$ per unit area. With the tetraploid epidermis the corresponding averages were $8{\cdot}0 \pm 0{\cdot}15$ and $7{\cdot}6 \pm 0{\cdot}15$. In both analyses the stomatal frequency per unit area was slightly greater when the underlying mesophyll was tetraploid but neither difference was significant. It is evident that

the number of stomata per unit area of the leaf cannot be used to distinguish periclinal chimeras in cacao.

REVERSION TO THE DIPLOID CONDITION.

Plants that have had some of their cells changed to the tetraploid condition by colchicine treatment are liable to revert to the diploid state because of the customary slower division of tetraploid cells. By relating the sectoring of the epidermis of leaves to the phyllotaxy of cacao an attempt was made to trace tetraploid sectors of the axis during the growth of the plant. A study was made of stomatal size and frequency on the leaves of twelve plants that showed indications of sectoring. Stomatal measurements were made on either side of the midribs of successive leaves as they reached maturity. The first-formed leaves after treatment were not considered as they showed a complicated sectoring. On an average, measurements were taken from fifteen leaves of each plant, covering a period of three and a half months and beginning two months after treatment. The phyllotaxy of cacao is 3/8 and therefore the ninth and successive leaves should reflect the ploidy of the first and successive leaves if there has been no change in sectoring.

All the plants except two had lost the sectoring of the epidermis by the time of development of the ninth leaf, having changed to a completely tetraploid or diploid epidermis. The ninth and all subsequent leaves had a similar epidermis. In most plants several leaves before the ninth were also similar. This suggests that the change occurs still earlier but, as the effect could be due to an original sectoring of the main axis and not to a change, its interpretation is uncertain. With seven of the twelve plants considered, the epidermis had reverted to the diploid condition whereas in three others it was completely tetraploid. On those plants in which the epidermis had reverted there were more leaves with a tetraploid epidermis than there were leaves with a diploid epidermis on plants changing to the tetraploid state. This would suggest a tendency to revert to the diploid state but it could not be definitely determined with these plants because the original sectoring was unknown. To obtain an accurate picture it would be necessary to section the stem at each node and attempt to determine the extent of the sector on a basis of cell size. With the two exceptional plants in which sectoring had not been completely lost there was good evidence of reversion to the diploid state, although each had only developed ten leaves. The ninth and tenth leaves had a diploid epidermis whereas the first and second in each case had a partially or completely tetraploid epidermis.

It was also evident from the results that immediately after treatment there were many tetraploid sectors of varying sizes. The first three leaves of many of the plants revealed six sectors.

The results given above cover six months in the life of the plant. In no plants of a greater age has any sectoring been found. In the study of leaf shape and thickness mentioned earlier, which was carried out on the youngest mature leaves of plants more than a year old, there was no evidence of epidermal sectoring nor were any half-leaf mosaics found. The latter only occur when the mesophyll is sectored, indicating that a histogenic layer other than the one giving rise to the epidermis consists of diploid and tetraploid cells. It is evident that, in seedling cacao, periclinal chimeras but not sectorial or mericlinal chimeras are able to persist. If a histogenic layer is composed of tetraploid and diploid cells, it will change to one or the other but there is no evidence of it affecting any other histogenic layer.

OTHER *Theobroma* SPECIES.

Attempts to induce polyploidy in other *Theobroma* species were less successful than with cacao. It was also more difficult to determine with leaf squashes the effect of the colchicine treatment. Large hairs are borne on the leaves of *T. bicolor*, *T. speciosa* and *T. grandiflora* and these hinder the flattening of the cells. The hairs provide a means of determining the constitution of the epidermis but they conceal the stomata. None of these difficulties occurs with *T. microcarpa* in which the squashing of leaves is as successful as in cacao.

Five plants of *T. microcarpa* were treated and five months later four of them were found to have one or more branches with tetraploid cells. The trees had formed their first jorquette by this time. One of the plants had at the jorquette one branch diploid, a second a periclinal chimera and the third totally tetraploid. The leaves subtending these branches had the same type of epidermis as the branch. The stomata of a diploid epidermis measured $15 \cdot 3\mu \pm 0 \cdot 32$ long as opposed to $18 \cdot 5\mu \pm 0 \cdot 32$ in the tetraploid and were less frequent per unit area in the latter.

Nine plants of *T. bicolor* were treated. With each plant the main shoot died and was replaced by two axillary shoots both of which were mericlinal chimeras. Five months later, tetraploid cells were found in only two shoots and both of these were periclinal chimeras with a diploid epidermis. As in cacao, the leaves of these shoots were darker green and thicker, with more pronounced veins and a changed shape.

Thirteen plants of *T. speciosa* were treated. The first-formed leaves after treatment were distorted and leaf squashes showed tetraploid cells, but in all but one plant the shoot reverted to the diploid state. The one exception was a sectorial chimera. The shoot was cut off above a leaf which, in addition to the normal tetraploid characteristics, had much larger epidermal hairs. The axillary bud had not developed into a shoot by the end of this study. Eight plants of *T. grandiflora* were treated, but none developed any tetraploid features.

From an extensive hybridization programme between *T. cacao* and the related species, hybrid seedlings were produced only from the crosses *T. cacao* × *T. angustifolia* D.C. and *T. cacao* × *T. grandiflora*. Of fifty-eight *T. cacao* × *T. angustifolia* hybrids twenty-two were treated with colchicine at various concentrations. These hybrids were very slow growing and there was a high degree of mortality among both treated and untreated plants. Leaf squashes and stomatal counts were made four months after treatment. None had developed with tetraploid cells. Only two *T. cacao* × *T. grandiflora* hybrids reached a sufficiently large size to treat with colchicine. Both of these seedlings died within a month of treatment.

DISCUSSION.

It is evident that the colchicine technique used will readily induce a tetraploid state in *T. cacao* but that it is less successful with some of the related species. Treatment of the hybrids was also unsuccessful, probably because of the slow rate of growth of these plants.

Levan (1942) has stated conditions under which the induction of polyploidy in a species is likely to lead to an increase in yield; these are that the optimal range of chromosome number which he considers as characteristic of the species should not be exceeded, that fertility should not greatly influence yield, and that the growth rate should not be markedly decreased. From this initial study of cacao it seems probable

PLATE I

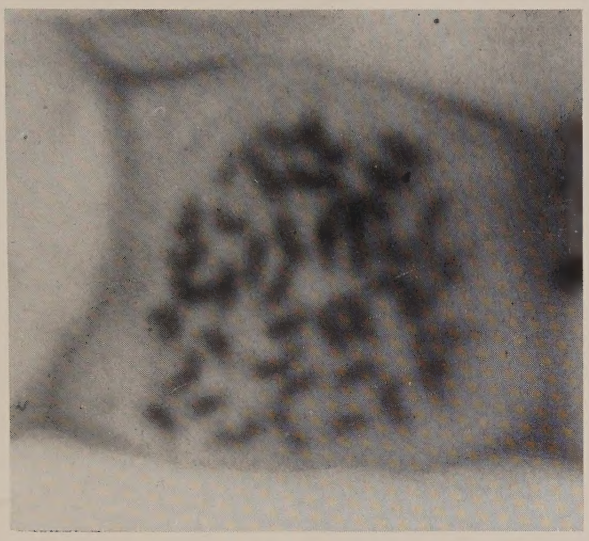

Fig. 1.
T.S. of a cacao flush leaf showing a tetraploid (4n = 40) epidermal cell.

Fig. 2.
Half-leaf mosaics of cacao with tetraploid mesophyll on the right and diploid mesophyll on the left of each leaf.

[*face p. 6*

that the first and third conditions are met. As to the second, bean development in cacao directly depends on compatible fertilization. Any irregularity in meiosis resulting from polyploidy would lead to a reduction in bean formation. The advantages of polyploidy in the tree might not be sufficient to compensate for this reduced fertility.

Periclinal chimeras are frequently produced in cacao as a result of colchicine treatment and these are able to persist. In the classical example of Winkler's *Solanum* chimeras, discussed by Neilson-Jones (1934), in which the plant consisted of tomato and nightshade tissues, the daughter seedlings reflected the tomato or nightshade nature of the generative layer of the parent. With the periclinal chimeras produced in cranberry by Dermen (1947), tetraploid seedlings were produced on selfing when the second histogenic layer was tetraploid, and diploid seedlings when it was diploid. This always occurred no matter what the degree of ploidy of the other histogenic layers might be.

Cacao is a cauliferous plant in which the flowers are borne on persistent islands of tissue known as cushions. Dade (1928) concluded, after mapping the cushions on a mature tree, that they had arisen in two ways: one series had developed in the axils of the leaves while several other series had arisen adventitiously and independently of the leaf traces. The development of individual cushions in a young tree was not studied. Adventitious buds in most plants develop from the stelar tissue and hence from the third histogenic layer. If the generative cells of the axillary cushions arise from the second histogenic layer and the adventitious cushions from the third histogenic layer, flowers differing in their degree of ploidy may be produced by the periclinal chimeras. The cacao chimeras produced as a result of colchicine treatment will, in a few years' time, provide material from which it will be possible to substantiate or invalidate the hypothesis of cushion development and its possible relation to fertility.

SUMMARY.

(1) A colchicine method is described for inducing polyploidy in *Theobroma cacao* and related species. A concentration of 0·6 per cent. in agar was found to be optimal in treating apical buds. Treatment was made when the cotyledons first opened to reveal the young shoot and axillary buds. Translocation of the colchicine was shown to occur in several instances.

(2) The tetraploid condition in cacao results in larger stomata, lower stomatal frequency per unit area, changes in leaf shape, leaf thickness, leaf colour and vein size.

(3) Attempts to distinguish periclinal chimeras on a basis of stomatal frequency alone were not successful.

(4) Polyploid sectors of the main axis, found in some cases up to six months after treatment, are not evident in the stem developing subsequently. These sectors are replaced by completely diploid or completely tetraploid layers. Periclinal chimeras, but not sectorial or mericlinal chimeras, are able to persist in the seedlings.

(5) Induction of polyploidy in related *Theobroma* species was less successful. Periclinal chimeras were produced in *T. microcarpa* and *T. bicolor*. Treatment of species hybrids was unsuccessful, probably because of their slow growth.

(6) The possible effects of polyploidy on the yield of cacao and the importance of periclinal chimeras if polyploidy results in a reduction of fertility are discussed.

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EFFECT OF THE EARLY ENVIRONMENT ON DEVELOPMENT OF FLOWERING IN THE TOMATO

I. TEMPERATURE

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THE effect of temperature on the growth and fruiting of the tomato, *Lycopersicon esculentum*, was studied by Went (1944, 1945). As a result of this work much is known of the responses of the tomato to changing temperature conditions. However, Went's temperature treatments were not given until the plants were several weeks old, so that there was no indication of the response to temperature treatments given during the first few weeks from germination.

Lewis (1953a) has shown that high temperature during and after germination increases the frequency of rogues in rogue-producing varieties. He also showed (1953b) that, shortly after cotyledon expansion, the plant enters a sensitive phase when variations in temperature will modify the number of flowers in the first inflorescence.

Roodenburg (1947) has referred to the effect of temperature on the number of leaves which appear before the first inflorescence and shown that, with a low temperature, a minimal number of leaves is formed. Goodall (1936, 1937) has also made observations on the position of the inflorescence.

The object of this investigation was to explore further the effects of temperature on the development of the plant.

MATERIALS AND METHODS.

In most of the experiments to be described, the plants were grown in two controlled environment rooms (C.E. rooms) for the first few weeks from cotyledon expansion. Later they were transferred to glasshouses where they were randomized on the benches.

In the C.E. rooms illumination was supplied by "Daylight" fluorescent lamps and the temperature control at plant level was $\pm$ 1° F. A description of the rooms has been recorded by Darlington (1951).

Different temperatures but equal light intensities were maintained in the two rooms. At specified times the plants were transferred from room to room. Seeds were germinated at a temperature of 65° F. and, as soon as the cotyledons had become horizontal, the seedlings were pricked out into 3½ inch pots containing the John Innes potting compost No. 1 (J.I.P.1).

RESULTS.

Inflorescence position.

In Experiment 1, temperature treatments extended over the first five weeks from cotyledon expansion. The temperatures of the C.E. rooms were as follows:

Low; 60° F. day, 50° F. night,
High; 80° F. day and night.

These temperatures were not chosen by the author but were those being used for other experiments running concurrently. The day-length was 16 hours and the average light intensity at plant level was 700 lumens/sq. ft. The terms "day" and "night" are used throughout this paper to indicate light and dark periods in the C.E. rooms.

Seeds of two varieties, Potentate and Ailsa Craig, were sown on 16th June 1953 and were pricked out and placed in the C.E. rooms on 25th June. There were ten treatments per variety. Treatments 1 to 5 started with periods of 1 to 5 weeks of low temperature, followed by periods of 4 to 0 weeks of high temperature, respectively. Conversely, treatments 6 to 10 started with high temperature periods followed by low. Afterwards all the plants were grown in a glasshouse until it was possible to count the number of leaves between the cotyledons and the first inflorescence. There were eight plants per treatment. The results are summarized in Fig. 1.

It will be seen that variations in temperature during the first two or three weeks from the pricking-out stage have caused variations in leaf numbers between the cotyledons and the first inflorescence, low temperature decreasing and high temperature increasing the number of leaves. This effect is shown clearly in each series for both varieties. There are, however, two interesting differences in the responses of the two varieties. Firstly, the range of leaf numbers is much greater for the variety Ailsa Craig where variations between 8 and 16 leaves were recorded over the whole series of treatments. The range for Potentate was 7 to 11 leaves. Secondly, the period of sensitivity is sharply defined for the variety Potentate, being limited to the first two weeks after pricking out; with Ailsa Craig it extends into the third week.

In Experiment 2, one C.E. room was set to give high day and low night temperatures (70° F., 50° F.) while the other provided the converse (50° F., 70° F.). The day-length was 12 hours and the light intensity was 700 lumens/sq. ft. Thus, all the plants received the same mean daily temperature during the whole experiment. Seeds were sown on 27th September 1954 and temperature treatments began on 8th October. There were thirty-six plants per treatment.

TABLE I.

Mean number of leaves to the first inflorescence (Exp. 2).

Temperature, ° F., over first three weeks		Potentate	Ailsa Craig
Day	Night		
70	50	8·11	8·64
50	70	8·28	8·83
Standard errors of means		±0·21	±0·26

The results, summarized in Table I, show that leaf numbers were the same whether high or low temperature occurred during the day or night. The controlling factor would appear to be the mean of the day and night temperatures. A similar conclusion was reached by Lewis (1953b) in relation to the temperature-sensitive period for flower number.

Effect of pre-germination temperatures.

In Experiment 3, seeds of variety Potentate were placed on moistened filter pads in petri dishes and kept at a constant temperature of 60° F. until approximately 50 per

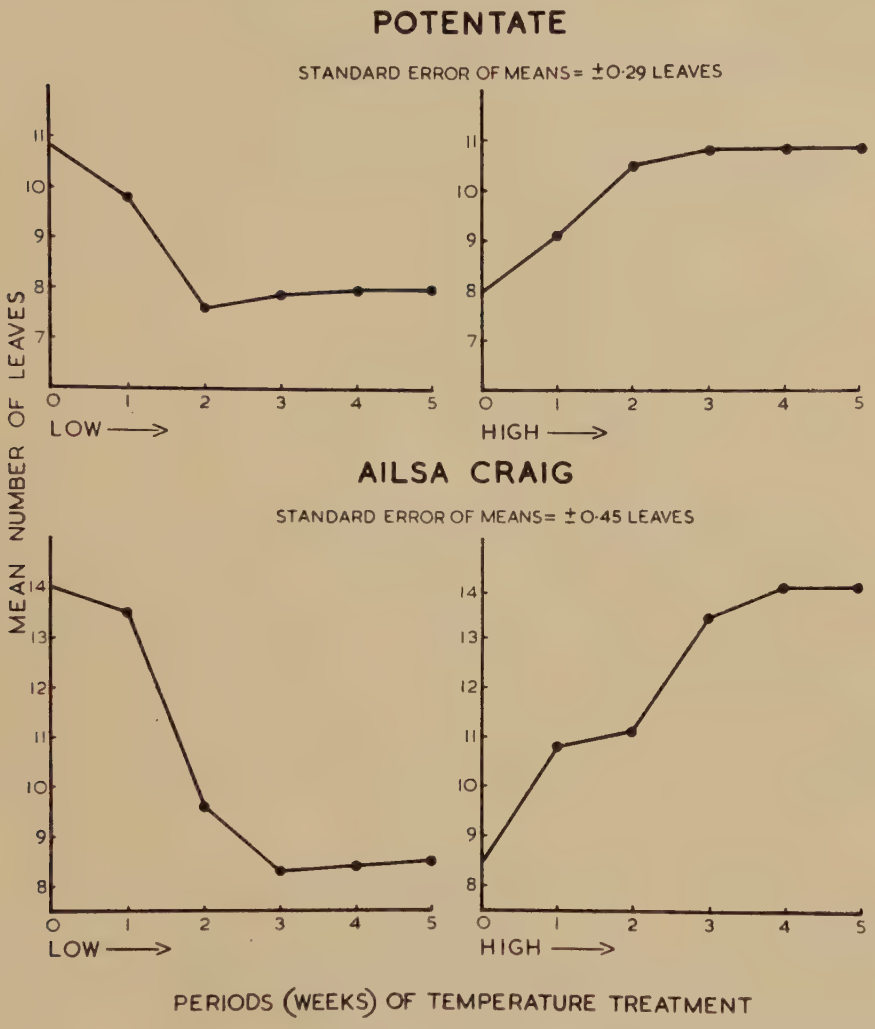

FIG. 1.

Effect of increasing periods of high and low temperature, beginning from cotyledon expansion, on the number of leaves to the first inflorescence.
High temperature; 80° F. day and night.
Low temperature; 60° F. day, 50° F. night.

cent. of the seeds had split their testas. At this point, temperature treatments as outlined in Table II began and continued for six days. All the seedlings were then pricked out and grown under glasshouse conditions until the number of leaves to the

first inflorescence could be counted. There were twelve plants per treatment. The results, summarized in Table II, show that no significant differences in leaf numbers have been induced as a result of temperature variations.

TABLE II.

Effect of varied temperature treatments on variety Potentate (Exp. 3).

Treatment	Mean number of leaves to first inflorescence
60° F. for 6 days	9·83
42° F. for 3 days 60° F. for 3 days	9·42
42° F. for 6 days	9·50
80° F. for 3 days 60° F. for 3 days	9·50
80° F. for 3 days 42° F. for 3 days	9·92
Standard error of means	±0·19

Experiment 4 was undertaken to test the response of seeds which had been soaked for twenty-four hours at room temperature and then subjected to increasing periods at 36° F. The seeds were soaked on moistened filter pads in petri dishes and later placed in a refrigerator for 10, 20 and 30 days. Control seeds were soaked for twenty-four hours prior to the termination of each cooling treatment so that treated and control seeds from each batch would germinate at the same time. This proved to be the case for seeds receiving 10 and 20 days' cooling but germination of seeds cooled for 30 days was retarded by about 2 days. Thirty plants per treatment were grown until leaf and flower numbers were counted. The results are summarized in Table III. No significant variations in leaf or flower numbers occurred between control and treated plants.

TABLE III.

Effect of pre-cooling treatments on development of tomatoes (Exp. 4).

Treatment	Mean number of leaves		Mean number of flowers	
	Potentate	Ailsa Craig	Potentate	Ailsa Craig
10 days at 36° F.	8·50	8·93	8·53	9·20
Control	8·37	8·93	9·40	9·03
20 days at 36° F.	8·13	8·70	8·97	9·83
Control	8·40	9·00	8·87	9·70
30 days at 36° F.	7·80	8·57	10·37	9·77
Control	7·83	8·27	10·63	10·67
Standard errors of means	±0·28	±0·34	±1·15	±0·94

Inflorescence size.

Lewis (1953b) has reported that between the eighth and twelfth day after cotyledon expansion the tomato is sensitive to temperature. During this period a low temperature causes the plant to initiate an increased number of flowers in the first inflorescence. From this evidence and the results of Experiment 1, it would appear that after cotyledon expansion the plant first becomes sensitive for the leaf number effect and immediately afterwards for inflorescence size.

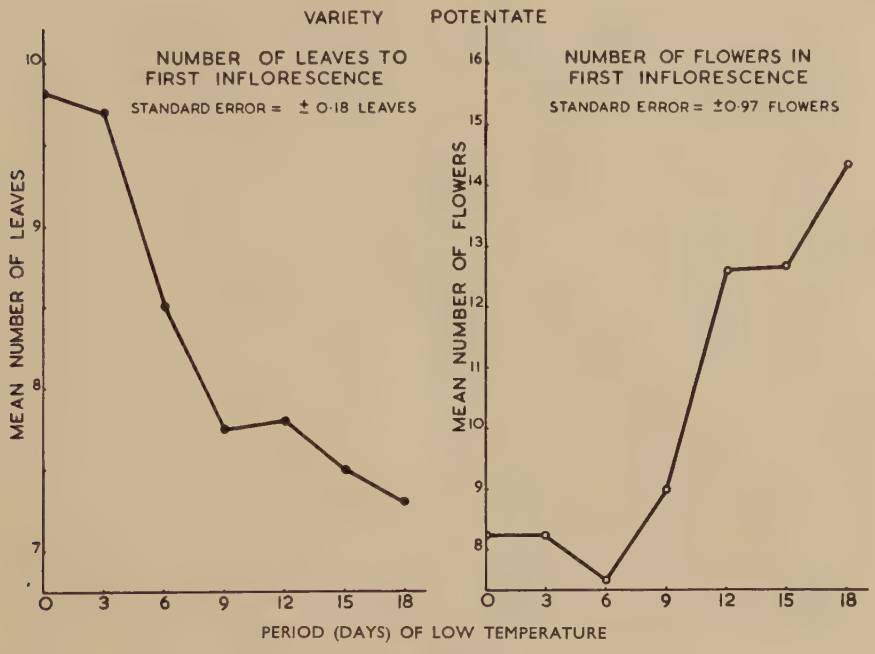
VARIETY POTENTATE
NUMBER OF LEAVES TO FIRST INFLORESCENCE
STANDARD ERROR = ± 0·18 LEAVES
MEAN NUMBER OF LEAVES
NUMBER OF FLOWERS IN FIRST INFLORESCENCE
STANDARD ERROR = ±0·97 FLOWERS
MEAN NUMBER OF FLOWERS
PERIOD (DAYS) OF LOW TEMPERATURE

FIG. 2.

Effect of increasing periods of low temperature (60° F. day, 50° F. night), beginning from cotyledon expansion, on the number of leaves to first inflorescence (left) and number of flowers in first inflorescence (right).

In Experiment 5, the unit low temperature period was reduced from one week to three days, thus: 3 days low temperature (60° F. day, 50° F. night), then 18 days high temperature (75° F. day, 65° F. night); 6 days low, then 15 days high; and so on. This was to enable the sensitive phases for leaf and flower numbers to be defined more accurately. Seeds were sown on 15th January 1954 and temperature treatments began on 25th January. The day-length was 12 hours and the mean light intensity 700 lumens/sq. ft. There were twelve plants per treatment. The results are summarized in Fig. 2. It is clear that, for the first nine days from cotyledon expansion, the plant is sensitive to temperature for the leaf number effect but not for flower number and that, from the ninth to the twelfth or fifteenth day, it is sensitive for the flower number effect but not for leaf number.

Experiment 6 was undertaken to determine the effect of temperature on the

second and third inflorescences. Seedlings were given increasing periods of cool treatment up to a maximum of six weeks from cotyledon expansion. The control temperature was 60° F. day and night and for the cool treatment the night temperature was reduced to 50° F. with the day at 60° F. The day-length was 10 hours and the mean light intensity was 700 lumens/sq. ft. Seeds of Potentate and Ailsa Craig were sown on 15th September 1952 and pricked out on 26th September. There were

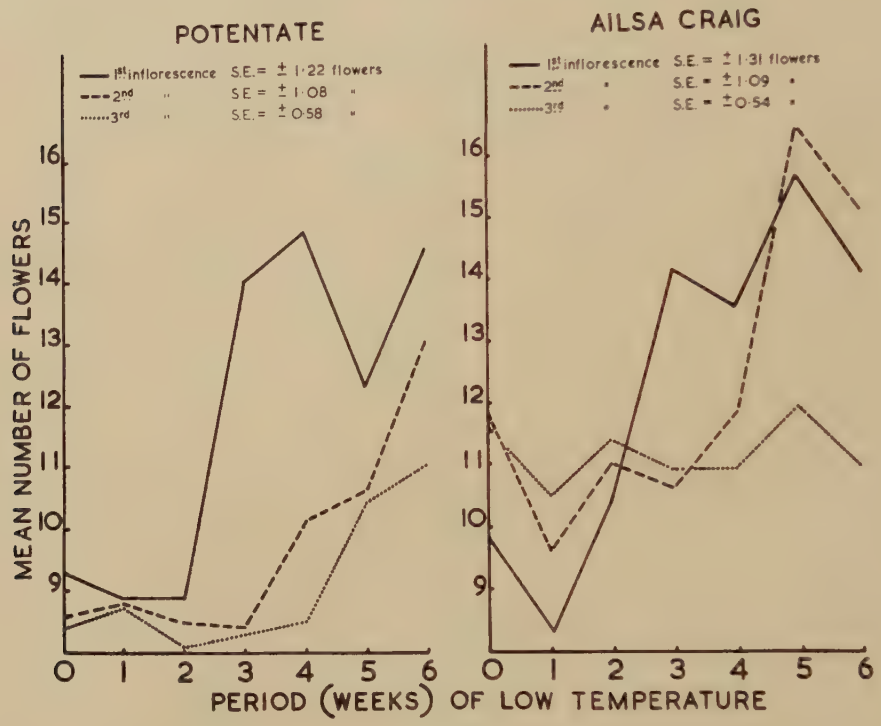
POTENTATE
AILSA CRAIG
1st inflorescence S.E.= ± 1·22 flowers
2nd " S.E. = ± 1·08 "
3rd " S.E.= ± 0·58 "
1st inflorescence S.E.= ± 1·31 flowers
2nd " S.E. = ± 1·09 "
3rd " S.E. = ± 0·54 "
MEAN NUMBER OF FLOWERS
PERIOD (WEEKS) OF LOW TEMPERATURE

Fig. 3.

Effect of increasing periods of low temperature (60° F. day, 50° F. night), beginning from cotyledon expansion, on the size of the first three inflorescences.

eight plants per treatment. At the end of six weeks all the plants received a constant temperature of 60° F. and grew at this temperature until the number of flowers in the first three inflorescences could be counted. To enable growth to proceed without starvation, the plants were potted-on at the appropriate time. The results are summarized in Fig. 3.

With Potentate, flower numbers for the first three inflorescences were increased when low temperature treatment was extended to six weeks from cotyledon expansion. Also, it would appear that the intervals between the temperature-sensitive phases for the first three inflorescences were about one week in each case. With Ailsa Craig, only the first two inflorescences showed a significant increase in flower number and the interval between their sensitive phases was two weeks.

PLATE I

Fig. 4.

Two plants of variety Ailsa Craig from Experiment 1, showing number and position of leaves.
Warm; 80° F. day and night.
Cool; 60° F. day, 50° F. night.

[face page 14

DISCUSSION.

Since the publication of the early work on the influence of temperature on the developmental phases in plants, several attempts have been made to vernalize tomato seed (Goodall and Bolas, 1942; Stier, 1938; Turner and Burr, 1937), but there is no clear evidence that low temperature treatment of the seed modifies the life cycle. In Experiments 3 and 4 of this investigation treatment of the seed did not produce significant differences in the number of leaves to the first inflorescence.

In a recent paper, Junges (1954) claims that vernalization of tomato seed affects the number of flowers produced rather than the time of flowering. It is difficult to see how this can be so. In Experiment 4 (Table III), a low temperature treatment apparently identical with that given by Junges—viz. 30 days at 36° F. (3° C.) after soaking for 24 hours—did not produce an increase in flower number. Moreover, this investigation shows clearly that the sensitive phase for flower number does not begin until approximately the ninth day after cotyledon expansion. This is corroborated by the findings of Lewis (1953b).

Thus it is the seedling and not the seed which responds to vernalization. The term vernalization, in this case, refers to a low temperature treatment which hastens flower initiation and consequently shortens the life cycle.

An interesting feature of the results is the different response of the varieties Potentate and Ailsa Craig under identical environmental conditions. These can be summarized as follows:

1. The minimum number of leaves between the cotyledons and the first inflorescence was seven for Potentate and eight for Ailsa Craig but the maximum was eleven and sixteen respectively.
2. The period from cotyledon expansion to the beginning of the sensitive phase for flower number, i.e. the vernalization period, extended into the second week for Potentate and into the third week for Ailsa Craig (Fig. 1).
3. Under certain environmental conditions (Experiment 6), the sensitive phases for the first, second and third inflorescences occurred at weekly intervals for Potentate, but at somewhat longer intervals for Ailsa Craig (Fig. 3).

These three factors are important in determining varietal earliness of cropping. With Potentate, which is generally considered to be an earlier variety than Ailsa Craig, a shorter vernalizing period, a smaller response to non-vernalizing temperatures and the shorter period between the initiation of inflorescences would all contribute to earlier flowering and fruiting.

The results of Experiment 2 indicate that the mean of the day and night temperatures during the vernalization period determines the degree of the leaf number response. Since, in this experiment, there was a difference of 20° F. between the day and night temperatures, it would appear that a high day temperature has compensated for a low night one and vice-versa.

In discussing the practical implications of these findings it should be borne in mind that, while high temperature increases the number of leaves to the first inflorescence, the continuation of such conditions for a number of weeks will cause increased vegetative growth. This has the effect of hastening the development of the inflorescence after initiation. Such a situation is illustrated in Fig. 4. The plants are both Ailsa Craig from temperature treatments 5 and 10 of Experiment 1. All the leaves to the first inflorescence have been removed but their numbers and positions are

indicated. It will be noted that, despite subjection to different temperatures for the first five weeks from cotyledon expansion, both plants flowered and fruited at the same time.

Thus it is clear that the ideal temperature programme for the tomato during the first few weeks after cotyledon expansion should allow for the highest growth rate consistent with satisfactory development. Although the same light intensity was used for all experiments in the C.E. rooms, it was not possible to arrange the same day-length. While it is possible that there is interaction between temperature and some aspect of the light conditions, it is unlikely that this would affect the conclusions drawn from the results.

It is intended that the influence of light on leaf and flower numbers should form the subject of a further paper.

SUMMARY.

(1) During a period beginning at cotyledon expansion and ending approximately nine days later, tomato seedlings can be vernalized. The number of leaves to the first inflorescence is minimized with low temperature and maximized with high temperature.

(2) The mean of the day and night temperatures over a period of 24 hours determines the degree of the leaf number effect. It is possible that a high day temperature compensates for a low night temperature and vice-versa.

(3) No modifications of leaf or flower numbers occur when low temperature treatments are applied to the seed.

(4) On completion of the vernalization period the seedling becomes sensitive for the number of flowers in the first inflorescence. This period extends to the twelfth or fifteenth day after cotyledon expansion. Low temperature increases and high temperature decreases the number of flowers.

(5) At a constant temperature of 60° F. the variety Potentate becomes sensitive at weekly intervals for the number of flowers in the first three inflorescences; under the same conditions the intervals are somewhat longer for Ailsa Craig.

ACKNOWLEDGMENTS.

The author wishes to thank Mr. W. J. C. Lawrence and Dr. D. Lewis for their interest and help and Miss V. M. Tucker for assistance during the course of the investigation.

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STUDIES OF FRUIT DEVELOPMENT IN RELATION TO PLANT HORMONES

IV. ACIDIC AUXINS AND GROWTH INHIBITORS IN LEAVES AND FRUITS OF THE APPLE

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SYNOPSIS.

Apple leaves and fruits are shown to contain three acidic growth-promoting and two acidic growth-inhibiting substances which appear to be different from any chemically known auxins or growth-inhibitors. By means of the technique of paper chromatography the distribution of these substances in fruits and seeds at different stages of development has been investigated. On the basis of the results so far obtained the possible significance of certain of these substances in fruit development is discussed.

During recent years much evidence has accumulated that the initiation and control of fruit growth depends on the action of specific chemical substances of a hormone nature produced at meristematic centres in the seed, and possibly also in the fruit tissue itself and in the adjacent leaves. Amongst these hormones the auxins, which control cell extension, are undoubtedly of first importance, since cell extension is the dominant process involved in the growth of fruits.

In spite of great advances made in the study of synthetic growth regulators our knowledge of these naturally occurring auxins remains extremely meagre and it is only recently that developments in technique, particularly the application of chromatographic methods, have made possible the detailed study of these substances. Results already published indicate that such studies are likely to be considerably more complex than was formerly imagined, for it appears that growth is controlled, not by a single auxin, but by a diversity both of growth-promoting and of growth-inhibiting substances. Thus, Wright (20) has reported three auxins and one inhibitor in black currant fruits; Luckwill (12) found four auxins and two inhibitors in apple seeds and similar results were obtained by Hancock and Barlow(4) in preliminary studies of apple leaf auxins. In extracts of strawberry achenes Nitsch (15), using chromatographic methods, has recently detected no less than seven different growth substances. The present paper describes experiments carried out between 1951 and 1955 to study the naturally occurring growth regulators of the apple and their distribution in leaves, fruits and seeds at different stages of development. Attention is confined to those substances which show activity in standard auxin tests and which occur in the acidic fraction of ether extracts. It is hoped to present a similar analysis of the non-acidic fractions in a subsequent publication. These experiments form part of a general investigation into the role of plant hormones in fruit development, previous contributions to which have appeared in this journal.

* Part of this work was carried out in the Pomology Department, Cornell University, N.Y., where the author held a Visiting Professorship from July 1954 until July 1955.

LEAF AUXINS.

Preparation and chromatography of leaf extracts.

During preliminary work it was observed that crude water or ether extracts of apple leaves were highly active in stimulating root formation on the hypocotyls of bean seedlings (*Phaseolus vulgaris*), and fractionation of such extracts by partitioning between ether and aqueous sodium bicarbonate showed that this auxin-like response resided in both the acidic and the non-acidic fractions. The nature of the substances responsible for the root-inducing properties of the acid fraction was investigated further by chromatographic analysis using the methods described below.

Samples of fully grown apple leaves of the variety Cortland were collected at various times between August and November from the terminal shoots of vigorous trees. The samples were dried at 70° C., ground to powder and thoroughly mixed. 1,200 gm. of this bulk sample was extracted with dry A.R. grade ether in a Soxhlet-type extractor for five hours. The ether was distilled off, leaving a dark green fatty residue which was then extracted eight times with 25 ml. portions of distilled water at 40° C. The combined water extract was shaken six times with its own volume of ether, each ether extract being kept separate and reduced by distillation to 75 ml. Each of these six ether extracts was shaken with 10 successive portions of 5·0 ml. 8 per cent. sodium bicarbonate, using the counter-current partition principle. This procedure was adopted to ensure a complete separation of the acidic and non-acidic fractions. The bicarbonate layers, containing the acidic fraction, were bulked, acidified to pH 4·0 with tartaric acid (using universal indicator paper) and extracted with four successive lots of ether. The ether extract was dehydrated over anhydrous sodium sulphate and taken to dryness by drip distillation in a small specimen tube.

The dry residue containing the ether- and water-soluble acidic substances from 1,200 gm. dry weight of leaves was dissolved in 0·1 ml. of ethanol and the alcoholic solution placed in a series of drops along the starting line of a chromatogram run on a large sheet of Whatman No. 1 filter paper. On the chromatogram sheet were also placed drops of leaf extract to which 1 μg. each of the synthetic auxins indoleacetic acid (IAA) and indolebutyric acid (IBA) had been added. These acted as marker spots.

After equilibration for several hours the chromatogram was developed in water-saturated butanol/ammonia solvent prepared by shaking together 100 parts each of butanol and water, and 8 parts by volume concentrated ammonium hydroxide. After 13 hours, when the solvent front had reached a point about 11 inches from the starting line, the paper was removed from the chromatography tank and dried. It was then cut into 21 transverse strips, each corresponding to a 0·5 division of the R_F scale, and the substances present on each strip eluted by irrigation with 3·0 ml. 90 per cent. ethanol. A previous test of this method of elution gave a 98·5 per cent. recovery of IAA as estimated on the spectrophotometer after treatment with Gordon and Weber's reagent (3). A portion of the chromatogram was not eluted with alcohol but was retained for examination under ultra-violet light and subsequent spraying with Ehrlich's reagent (p-dimethyl-aminobenzaldehyde in conc. hydrochloric acid diluted with four volumes of acetone before use) to detect indole derivatives.

Biological testing of chromatograms.

Samples of the alcoholic eluates from the different portions of the chromatogram were tested for auxin activity in the bean rooting test (13), the wheat coleoptile

cylinder test (9, 20) and the *Coleus* petiole abscission test (13), by the methods described in previous publications. These three tests were used because each measures a different type of auxin response, viz. root initiation, cell extension and delay of abscission, respectively. Where the extract was required in aqueous solution, as for

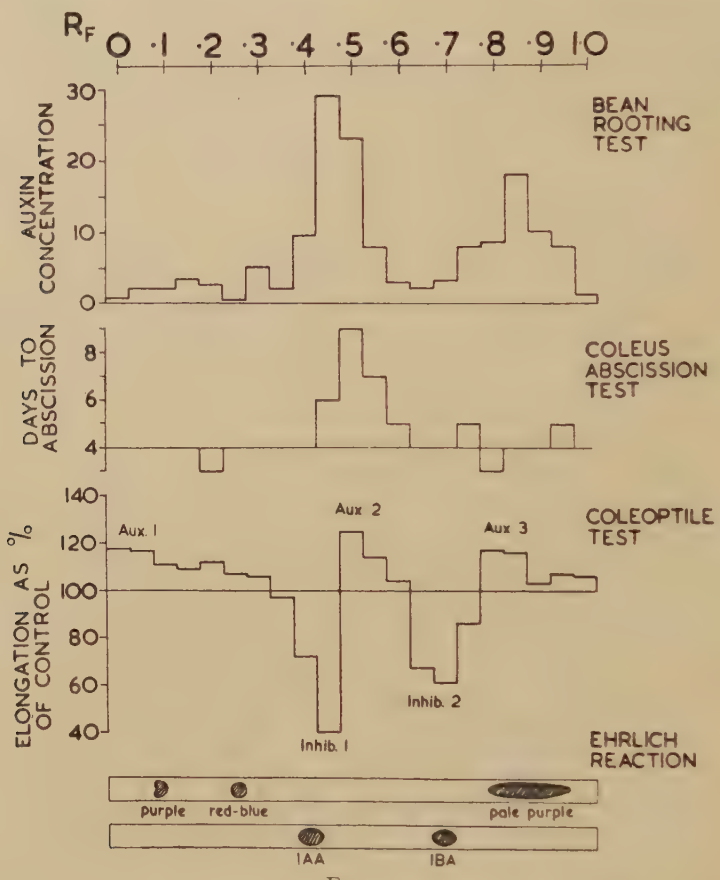

FIG. 1.

Apple leaf. Paper chromatogram of acidic fraction of ether extract divided into 21 portions and assayed in three different tests. Concentrations used were: Bean test, 114 gm. dry wt. leaf tissue per ml. of extract; *Coleus* test, 114 gm. per petiole; Coleoptile test, 38 gm. per ml. Below is shown the location of Ehrlich reactors and of synthetic IAA and IBA on parallel chromatograms.

the rooting and coleoptile tests, the necessary amount of alcoholic solution was evaporated to dryness and the residue digested with the required quantity of distilled water.

The results of the three biological tests on this chromatogram are presented in Fig. 1 in the form of histograms, in which biological activity is plotted against R_F value. The horizontal line in each diagram represents the level of activity in the controls treated with distilled water only. For the bean rooting test, biological activity, measured as the mean number of root primordia initiated on 20 cuttings,

has been transformed into relative auxin concentration by using a previously constructed concentration-response curve. In the *Coleus* test each mean is based on the response of 20 replicate petioles and in the coleoptile test on the mean elongation of 10 coleoptile sections (initial length 2 mm.) over a period of 24 hours. The variability of the material in the latter test was such that a difference from the control of 15 per cent. is required for significance at the 5 per cent. level of probability. In the coleoptile test each extract was tested at a number of different dilutions in order to ensure that the growth inhibitions noted were not due to supra-optimal concentrations of auxin.

The chromatographic analysis (Fig. 1) shows clearly that two separate substances contribute to the root-inducing properties of the acidic fraction. These same two substances appear to be active also in stimulating cell extension growth in coleoptiles, and one of them (at R_F 0·5) has in addition the property of delaying abscission in *Coleus* petioles. The coleoptile test also reveals three further substances with marked effects on cell elongation; one of these is a growth-promoting substance which moves only a short distance from the starting point ($R_F = 0·1$), whilst the other two ($R_F = 0·4$ and $R_F = 0·7$) are growth inhibitors; none of these three substances appears to have any appreciable activity in the *Coleus* abscission or bean rooting tests. For ease of reference, and to distinguish them from other biologically active substances recently reported from the black currant, these substances will be provisionally referred to as *Malus* auxins 1, 2 and 3, and *Malus* inhibitors 1 and 2.*

It will be noticed that, in the coleoptile cylinder test, the response of auxin 2 is partially masked by inhibitor 1, which overlaps it on the chromatogram. It is possible that inhibitor 2 also is interfering with auxin response in this test. The bean rooting test, being insensitive to these inhibitors, thus gives a much clearer picture of the auxin responses and would obviously be the preferable method to use in any quantitative investigation. The effects of these cell-extension growth inhibitors is shown most clearly by fraction 10 ($R_F = 0·45$), which gave a positive response in the rooting and abscission tests, yet in the coleoptile test produced strong inhibition of growth.

Concentration of acidic fraction by adsorption on alumina.

In view of the observation of Linser (7) that several naturally occurring acidic growth regulators could be adsorbed on alumina from alcoholic solution, an attempt was made to concentrate the acidic auxins and inhibitors of the apple leaf in this way, as an alternative to the method already described (p. 19).

700 gm. of dried and powdered leaves of the variety Cortland were placed in a glass cylinder (10 cm. diameter × 40 cm. long) and 1·5 l. of absolute ethanol was allowed to percolate slowly through the column of leaf powder. The extraction process lasted 5 hours. The alcoholic extract was then passed through a column of activated alumina (2·5 cm. × 13 cm.) which was afterwards washed with 100 ml. of ethanol, dried and extracted with 100 ml. of 4 per cent. sodium bicarbonate. The bicarbonate solution was neutralized, brought to pH 4·0 with tartaric acid, and extracted three times with three times its volume of A.R. ether. The ether was dehydrated with anhydrous sodium sulphate and taken to dryness by drip distillation. The extract was taken up in 0·05 ml. ethanol and analysed chromatographically on Whatman No. 1 filter paper, using the butanol/water/ammonia solvent previously described. Some chromato-

* In subsequent references to these substances in the text and illustrations the word *Malus* is omitted except where its absence might lead to uncertainty.

grams were bioassayed by the wheat coleoptile cylinder test, whilst other parallel chromatograms were sprayed with Ehrlich and Salkowski reagents and examined under ultra-violet light. The results of a typical experiment with this extract are given in Fig. 2.

It is clear from the results of the coleoptile test that, with the possible exception of inhibitor 1, the same biologically active substances are present in this extract as in the extract prepared by the previous method. Although their estimated R_F values are somewhat different, because of the use of a smaller chromatography tank, auxins

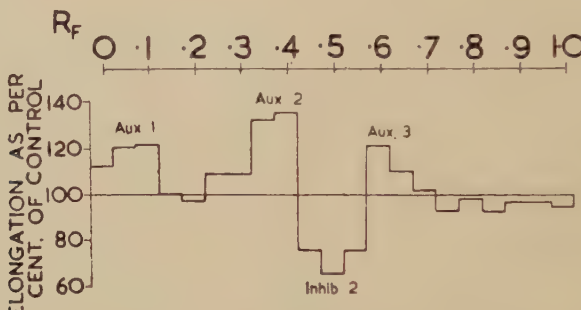

FIG. 2.

Apple leaf. Paper chromatogram of acidic fraction extracted with ethanol and concentrated by adsorption on activated alumina. Assayed in wheat coleoptile test. Concentration equivalent to 20 gm. dry wt. leaf tissue per ml. of extract.

1, 2 and 3 and inhibitor 2 are clearly recognizable from their relative positions on the chromatogram.

FRUIT AND SEED AUXINS.

EXTRACTION PROCEDURE FOR FRUITS AND SEEDS.

The drying of material by heat prior to extraction, as was done for apple leaves, is open to the objection that certain of the biologically active substances may have been destroyed by the heating or, alternatively, that certain of the active substances found might be artefacts produced as a result of this treatment. Consequently, in the study of auxins and inhibitors in the seeds and fruit tissue of the apple, the ether extracts were prepared from freshly gathered material without previous drying. In order to suppress enzyme activity during extraction, particularly the possible enzymatic conversion of precursors into free auxin, the extraction was carried out at just above freezing point and the period of extraction was limited to four hours.

For the extraction of auxins from fruits, 300-500 gm. of tissue were passed quickly through a domestic mincing machine and the minced tissue allowed to fall directly into a beaker of cold ether. Sufficient ether was used to cover the tissue completely and powdered thiourea, at the rate of 0·1 gm. per 100 ml., was added to inhibit the enzymatic browning of the tissue. Preliminary experiments had shown that the bulk of the thiourea was removed during the subsequent purification of the extracts and that the amount left in the final residue did not interfere in any way with the biological tests. Small seeds were extracted by placing them directly in ether, with-

out the addition of thiourea; larger seeds were extracted in the same way except that they were first cut into halves or quarters to facilitate rapid penetration of the ether. At the end of the four-hour extraction period the plant material was separated from the extract by straining through glass wool. The acidic fraction was separated by shaking with sodium bicarbonate and was concentrated by the same method as was used for the leaf extracts. Where, as with apple seeds, the tissues were rich in phloridzin, relatively large quantities of this glucoside were frequently found in both the acidic and the non-acidic fractions. In order to remove the glucoside, the dried extract was dissolved in 1 to 2 ml. of hot water and then cooled to just above freezing point for several hours. Phloridzin, being practically insoluble in cold water, was precipitated on cooling and could be removed by filtration. The acidic substances were then recovered from the filtrate by further partitioning into ether.

During 1952 and 1953 many extracts were made of fruit buds, flowers, seeds and fruits of the apple varieties Stoke Red, Sweet Alford, White Jersey and Lord Lambourne at various stages of development. The acidic fractions of such extracts, prepared as described above, were analysed chromatographically in butanol/ammonia in the same way as the leaf extracts. Auxins and growth-inhibiting substances on the chromatograms were located by means of the wheat coleoptile cylinder test, each extract being tested at several different concentrations. A tabular summary showing the distribution of auxins and inhibitors in the various tissues examined is given in Table I and some typical chromatograms are illustrated in Figs. 3, 4 and 5. As in the previous experiments, only means differing from the control by at least 15 per cent. are significant at the 5 per cent. level of probability.

Auxins and inhibitors in the seed.

The seed of the apple shows three well-defined growth phases (8) which may be briefly defined as follows:

Stage 1, lasting from 2 to 4 weeks after petal-fall depending on the variety, is characterized by the free-nuclear endosperm, rapid growth of the nucellus but very slow growth of the embryo.

Stage 2 extends to the end of the "June drop" and is characterized by a cellular endosperm and very rapid growth and differentiation of the embryo.

Stage 3 is the maturation phase during which reserve products accumulate in the embryo but there is little further growth.

Fig. 3 gives illustrative chromatograms prepared from extracts of seeds of different ages. The general pattern of auxins and inhibitors found during stage 1 was similar to that already described for leaf tissue. Auxins 1, 2 and 3 could be readily identified and inhibitor 1 was present in relatively large quantity. Inhibitor 2, on the other hand, appeared to be absent from the seeds at this early stage of development, or at least to be present only in very small amounts. With the onset of stage 2 the auxin pattern changed somewhat, auxin 3 being dominant throughout this phase of development, though traces of auxins 1 and 2 were also found on many chromatograms. Inhibitor 1, present in quantity at the beginning of stage 2, disappeared rather suddenly when the embryo was about half-grown. At about the same time inhibitor 2 made its appearance and increased in quantity as the embryo neared its final size. During stage 3, auxin 3 and inhibitor 2 were conspicuous features of most

TABLE I.

Auxins and growth-inhibitors detected on chromatograms of acidic fractions of ether extracts of apple by means of the wheat coleoptile cylinder test.

Extract Number	Variety	Organ or tissue extracted	Stage of development	Auxins			Inhibitors		Ref.
				1	2	3	1	2	
305	S.R.	Seed	Late stage 1	++	++	++	++	−	Fig. 3
307	S.R.	Seed	Early stage 2	+	+	++	++	?	
308	S.R.	Seed	Mid-stage 2	+	+	++	++	+	
269	L.L.	Seed	Stage 2	+	?	++	−	+	
271	S.A.	Seed	Stage 2	−	−	++	−	++	Fig. 3
317	S.R.	Seed	Stage 3	−	−	++	−	+	
320	S.R.	Seed	Stage 3	?	?	++	−	++	Fig. 3
278	S.A.	Embryo	Stage 2	?	+	+	−	?	
279	S.A.	Endosperm + testa	Stage 2	?	+	+	−	+	
282a	W.J.	Embryo	Stage 3	−	?	?	−	++	Fig. 3
282b	W.J.	Endosperm	Stage 3	−	++	?	?	+	
282c	W.J.	Testa	Stage 3	?	++	?	++	++	
288	W.J.	Embryo, after alk. hydrolysis	Stage 3	?	++	+	?	+	Fig. 3
283a	W.J.	Embryo	After-ripened 57 days	−	++	?	−	+	Fig. 3
283b	W.J.	Endosperm	After-ripened 57 days	−	−	−	−	+	
283c	W.J.	Testa	After-ripened 57 days	−	+	−	+	−	
285	W.J.	Embryo	After-ripened 89 days	−	++	−	−	?	
256	D.	Pollen	Full-bloom	?	?	?	++	++	
300	S.R.	Anthers	Full-bloom	−	+	−	++	++	
299	S.R.	Ovary	Full-bloom	−	−	−	−	++	
263	S.R.	Fruitlet	⅛ in.-¼ in. diameter	+	+	+	+	+	
302	S.R.	Fruitlet	¼ in. diam., 4 days P.F.	?	+	?	+	+	
267	S.R.	Fruitlet	¼ in.-1 in. diameter	?	++	?	+	+	
303	S.R.	Fruitlet	½ in. diam., 19 days P.F.	?	?	+	++	+	
304	S.R.	Fruitlet	26 days P.F.	−	++	−	++	+	Fig. 4
306	S.R.	Fruit flesh	42 days P.F.	−	++	−	+	+	
313	S.R.	Fruit flesh	77 days P.F.	−	++	−	?	++	Fig. 4
314	S.R.	Pedicel	77 days P.F.	+	++	+	++	+	
319	S.R.	Fruit flesh	Harvest ripe	?	++	−	−	++	Fig. 4
322	L.L.	Fruit flesh	Chat fruit	−	−	−	−	++	Fig. 5
323	L.L.	Fruit flesh	Normal fruit at harvest	−	++	?	−	++	Fig. 5
290	S.R.	Fruit bud	Swelling	−	?	−	++	−	
291	S.R.	Fruit bud	Bursting	−	++	?	+	++	
294	S.R.	Fruit bud	Mouse-ear	−	++	?	+	++	
296	S.R.	Fruit-bud leaf	Early green-cluster	−	+	−	++	++	
297	S.R.	Flower-bud	Early green-cluster	−	+	−	+	++	

KEY TO ABBREVIATIONS USED.

Varieties:
S.R., Stoke Red; L.L., Lord Lambourne; S.A., Sweet Alford; W.J., White Jersey; D., Dufflin.
Stage of development:
P.F., post-fertilization.
Abundance of auxin or inhibitor:
++, auxin or inhibitor present in relatively large amounts as judged by the size of the histogram peaks.
+, auxin or inhibitor present in smaller amount but sufficient to give a peak significant at 5% probability when tested at a favourable concentration.
?, auxin or inhibitor suspected as being present in small quantity but no significant peaks obtained on the histograms.
−, no trace of auxin or inhibitor found.

chromatograms, though auxin 2 was also present in some quantity in the endosperms and testas of mature seeds of White Jersey.

Dormant embryos contained inhibitor 2 but only traces of auxins 2 and 3. However, after subjecting dormant embryos to mild alkaline hydrolysis at pH 10 prior to extraction, a well-marked auxin 2 peak could be demonstrated (Fig. 3). Similar auxin 2 peaks were obtained from embryos taken from seeds which had been after-ripened at 4° C. for 30 to 60 days. These observations suggest that in the dormant embryos auxin 2 is present in some inactive or "bound" form from which it can be released either by alkaline hydrolysis or by natural enzymatic processes during after-ripening at low temperature. The appearance of auxin in apple embryos towards the end of the after-ripening period has been reported in a previous publication (10).

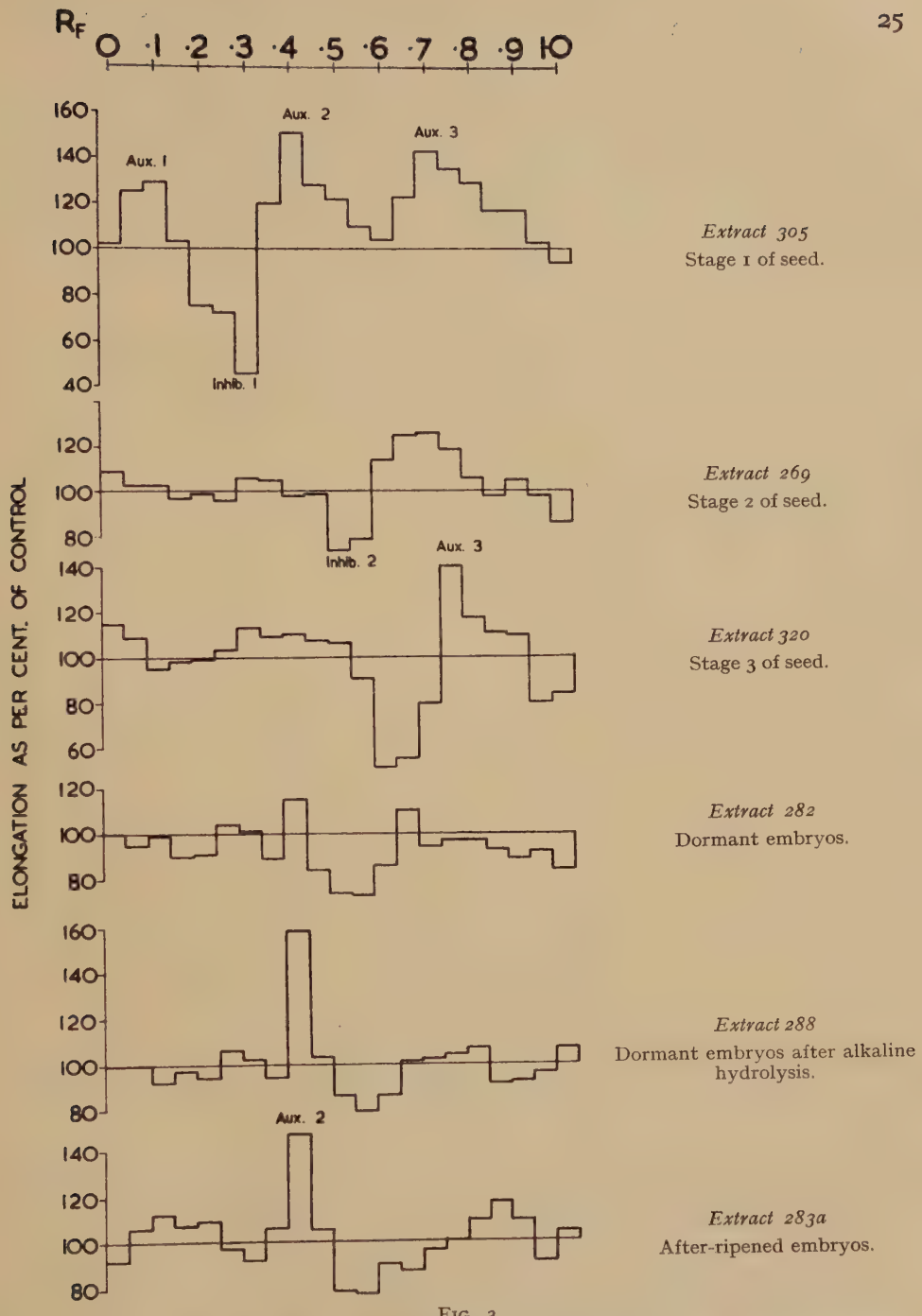

FIG. 3.

Apple seeds and embryos. Paper chromatograms of acidic fractions of ether extracts assayed in wheat coleoptile test. Concentrations used were: *Extract 305*, 240 seeds/ml.; *Extract 269*, 50 seeds/ml.; *Extract 320*, 25 seeds/ml.; *Extract 282*, 40 embryos/ ml.; *Extract 288*, 60 embryos/ml.; *Extract 283a*, 40 embryos/ml. See Table I for further details.

AUXINS AND INHIBITORS IN BUDS, FLOWERS AND FRUIT.

Fruit buds. Extracts of fruit buds were made at four stages of development, viz. "swelling", "bursting", "mouse-ear" and "early green-cluster". At the earliest stage of bud development inhibitor 1 was the only active compound which could be definitely identified on the chromatograms. At later stages auxin 2 and inhibitor 2 also became prominent. At "early green-cluster" small, though non-significant, peaks were obtained at R_F values corresponding to auxin 1 and auxin 3.

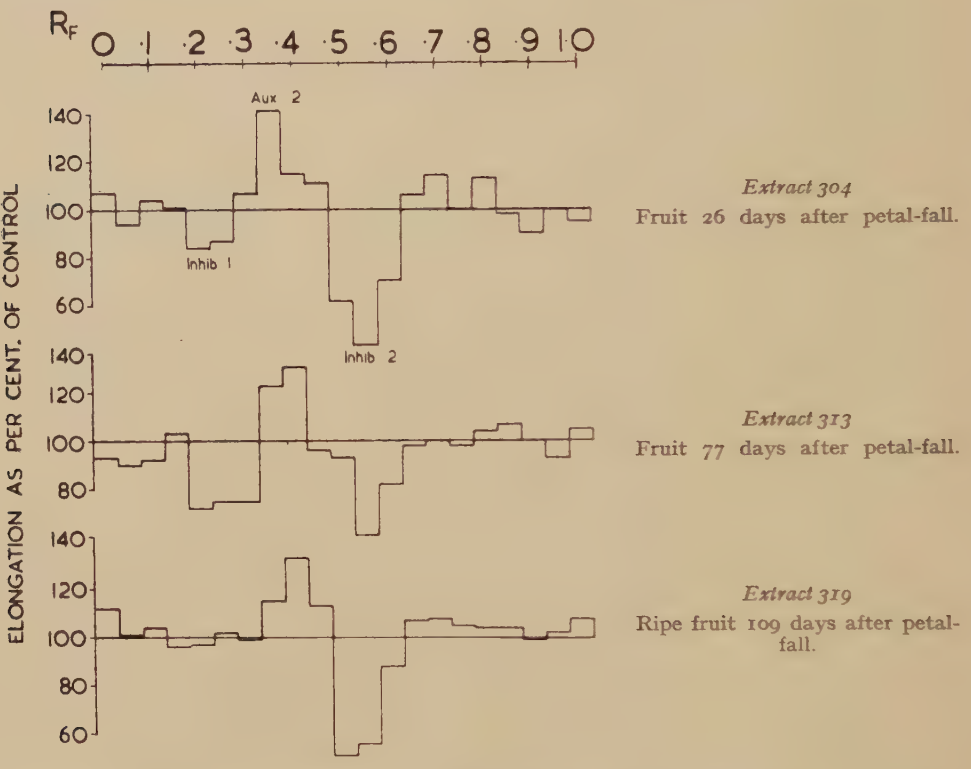

FIG. 4.

Apple fruit tissue. Paper chromatograms of acidic fractions of ether extracts assayed in wheat coleoptile test. Concentrations used were: *Extract 304*, 6 fruits/ml.; *Extract 313*, 2 fruits/ml.; *Extract 319*, 1 fruit/ml. See Table I for further details.

Flowers. Extracts of apple flowers prior to fertilization failed to show any auxin activity, though inhibitor 2 was present. Extracts of the ripe anthers showed auxin activity only at very high concentrations of the order of 3 gm. of anthers per chromatogram spot. Auxin 2 appeared to predominate, though auxins 1 and 3 were possibly present in small amounts as well. Larsen and Tung (5), using different techniques, have also demonstrated the presence of both acidic and neutral auxins in apple pollen, though in contrast to the pollen of certain other plants that of apple cannot be considered a particularly rich source of "free" auxin.

Fruit. The predominant auxin in the fruit flesh throughout its development was auxin 2 (Fig. 4). This was detected in fruits of Stoke Red as early as four days after petal-fall and was still present in the flesh of ripe fruits collected 105 days later. Traces of auxins 1 and 3 were sometimes observed on the chromatograms of fruit extracts, though these never reached a significant level. Both inhibitors were found in the growing fruits, but only inhibitor 2 appeared to to be present in the flesh of mature fruits.

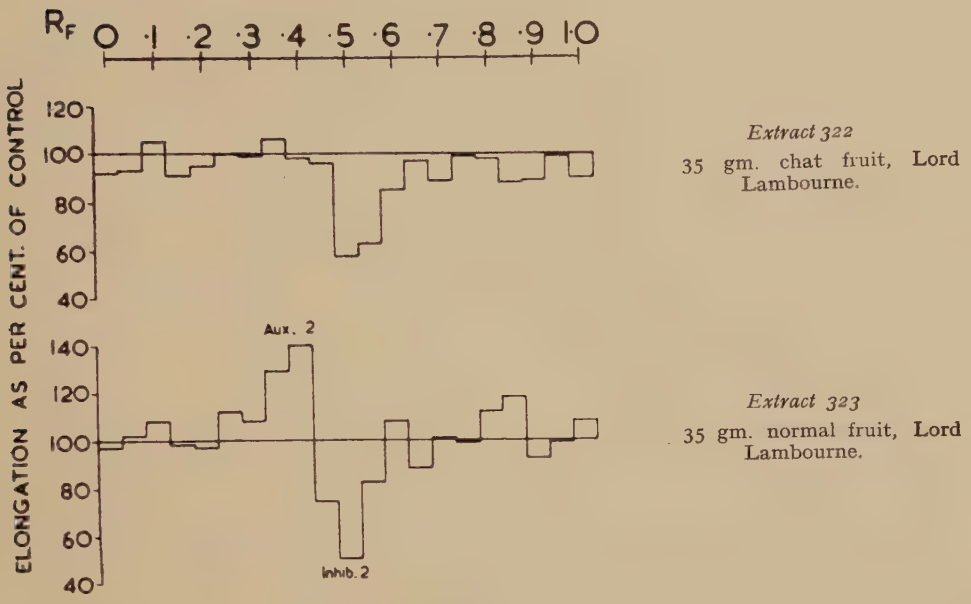

FIG. 5.

Extracts of normal fruit (below) and chat fruit (above) of Lord Lambourne apple, analysed chromatographically and tested in wheat coleoptile test at a concentration of 35 gm. fruit tissue per ml. of extract.

In contrast to the fruit flesh, the stalks of Stoke Red apples collected 77 days after petal-fall were found to contain all three acidic auxins and both inhibitors. This chromatogram, in fact, closely resembled those given by seed and leaf extracts.

In Fig. 5 a comparison is made between an extract of normal fruits of the apple variety Lord Lambourne and a comparable extract made from fruits infected with chat fruit virus. This virus causes the fruits to stop growing while they are still only 1 to 1½ inches in diameter and, although the seeds appear to develop normally, the fruit flesh remains hard and green until the end of the season. As will be seen from Fig. 5, auxin 2 is completely absent from such chatted fruits, although present in considerable quantity in the flesh of normal fruits collected at the same time from adjacent trees. This evidence suggests a connection between the presence of auxin 2 and the growth and ripening of the fruit.

CHARACTERISTICS OF GROWTH REGULATORS EXTRACTED FROM APPLE TISSUE.

Concentration-response curves in coleoptile test.

The experiments and observations reported in this section were made on samples of the biologically active substances collected by elution from the relevant zones of

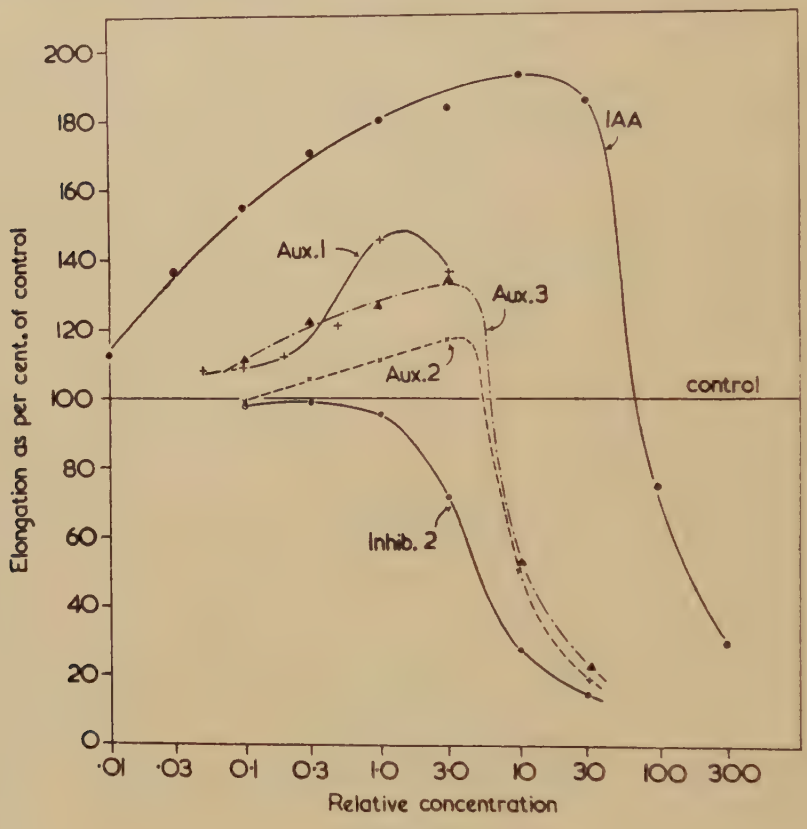

Fig. 6.

Comparative concentration/response curves in the wheat coleoptile cylinder test of *Malus* auxins 1, 2 and 3, *Malus* inhibitor 2 and synthetic IAA.

paper chromatograms of leaf, seed or fruit tissue. The elutions were made from relatively narrow zones of the chromatograms (5 to 10 per cent. of the total chromatogram length) but, owing to trailing and overlapping of the growth-promoting and growth-inhibiting substances, some contamination of the samples was inevitable.

In Fig. 6 the concentration-response curves for auxins 1, 2 and 3 and for inhibitor 2 are shown. The relative positions of these curves on the abscissa are arbitrary since there is no method of knowing the absolute concentrations of active material in the samples tested. It is clear, however, that all three of the natural auxins are very much less active in this test than is IAA, the optimum elongations induced being of the

order of 120 to 140 per cent. of the water controls, compared with 190 per cent. for IAA. Further, the range of concentrations over which they are active in inducing cell elongation is approximately 30 times smaller than the corresponding range for IAA. It seems unlikely that such large differences as these could be accounted for by contamination of the auxin samples with small quantities of inhibitory substances. Inhibitor 2 suppressed coleoptile growth over the whole range of concentrations tested, whereas auxins 2 and 3, like IAA, were inhibitory only at high concentrations.

Biological and chemical properties.

Auxin 1 (R_F 0·05-0·1 in butanol/ammonia) is a cell-elongation promoting substance which occurs rather spasmodically in extracts of leaf and seed tissue. It may also occur in small quantity in the fruit flesh. Its R_F value is similar to that of indolepyruvic acid, a labile precursor of IAA, which Stowe and Thimann (17) claim to have identified on chromatograms of sweet corn extracts. Apple chromatograms sprayed with Ehrlich's reagent frequently show a purple spot at this position (Fig. 1) which might indicate that the active substance is an indole derivative. However, its stability to light and heat make it unlikely that it can be identified as indolepyruvic acid. It may be identical with "accelerator α", a growth-promoting substance which Bennet-Clark and Kefford (1) found close to the starting line on their chromatograms of various plant extracts. Bloemmaert (2) also found activity at this position on chromatograms of ether extracts of potato peelings, and Hancock and Barlow (4) in apple leaves. The possibility must be considered that this substance is not a true auxin but merely an accessory substance which promotes growth through synergistic action with some naturally occurring auxin in the wheat coleoptile. The inactivity of this compound in other auxin tests (e.g. root initiation and petiole abscission) suggests that this may be so.

Auxin 2 (R_F 0·3 to 0·5 in butanol/ammonia) is the most characteristic and widespread growth-promoter in apple tissue. It is particularly abundant in the rapidly growing seeds during stage 1 of their development, in after-ripened embryos, in bursting buds and in the flesh of growing fruits, which suggests that it may be associated in the plant with the process of cell expansion. This view is supported by its absence from chat fruits and from the ovaries at the time of anthesis since in neither of these tissues is active cell expansion in progress. It is also relatively scarce in the seeds during stage 2, when most of the growth is attributable to cell division in the embryo rather than to cell expansion. However, the presence of auxin 2 in the mature leaves, in which both cell extension and cell division have long since ceased, must also be borne in mind. The activity of this substance in all three types of biological tests also suggests that it is a true auxin. It gives no colour reactions with either Ehrlich or Salkowski type reagents and shows no ultra-violet fluorescence. In both butanol and propanol its R_F value is about 10 per cent. higher than that of IAA and is intermediate between IAA and IBA (Fig. 1). When eluted and re-chromatographed in water according to the technique of Sen and Leopold (16) it runs with an R_F value of 0·5 to 0·6 and shows a clear separation from IAA at R_F 0·9-1·0. The evidence that this auxin is distinct from IAA is discussed in more detail in a previous publication (14).

Unknown acidic auxins with an R_F value slightly higher than that of IAA in butanol have been found by Wright in black currant fruits (20) and by Bloemmaert in potato skins (2). The auxin described by Wright was shown by its colour reactions

to be an indole compound and is therefore not the same as *Malus* auxin 2. Bloemmaert suggested that his auxin having an R_F of 0·4 to 0·42 might be IBA, but the R_F value, the colour reactions and the biological activity of *Malus* auxin 2 do not correspond with those of IBA. A further difference is that in black currant and potato the unknown auxins occur in association with IAA, whereas in the apple no evidence of IAA has been found, either in the leaves or in the fruit. Hancock and Barlow (4) found a growth-promoting substance in young apple leaves which had an R_F close to that of IAA and which was probably the same as that described in the present paper as *Malus* auxin 2. Their failure to find this auxin in older leaves may have been due to masking by inhibitory substances.

Auxin 3 (R_F 0·75-0·95 in butanol/ammonia) shows marked activity in the coleoptile extension and bean rooting tests, though not in the *Coleus* test. It is also active in the tomato parthenocarpy test and may probably be identified with the seed auxin reported in previous papers (8, 11) as being associated with the control of fruit drop in the apple. It appears to be a very weak Ehrlich reactor, indicating that it may be an indole compound, though no Salkowski reaction has been obtained with this auxin. *Malus* auxin 3 is probably identical with the acidic growth-promoting substance found by Hancock and Barlow (4) at R_F 0·8 in apple leaf extracts.

Inhibitor 1 (R_F 0·2 to 0·4 in butanol/ammonia) is a strongly acidic substance which can be detected by spraying the chromatogram with an indicator such as bromocresol green. At low concentrations it has little effect on coleoptile elongation but in larger doses it is toxic, causing bleaching and death of the sections. It differs in R_F value from malic and citric acids, the two commonest organic acids of apple tissue, and is also distinct from phloridzin which, as Thimann and Marré (19) have shown, inhibits growth of coleoptile sections. It is considered that this toxic substance is unlikely to have any biological significance as a growth regulator.

TABLE II.

Growth of wheat coleoptile sections in solutions of Malus *inhibitor 2, with and without the addition of 2 : 3-dimercapto-1-propanol (BAL) during 24 hours, and their subsequent growth in three days when transferred to IAA 3 p.p.m. Means of 30 sections of initial length 2 mm.*

Relative conc. of inhibitor	Conc. of BAL (p.p.m.)	Growth in 24 hours (mm.)	Subsequent growth after 3 days in IAA (mm.)	Total growth
0	0	1·33	0·15	1·48
0	25	1·40	0·29	1·69
5	0	1·08	0·45	1·53
5	25	1·08	0·51	1·59
10	0	0·97	0·41	1·38
10	25	0·96	0·53	1·49
25	0	0·80	0·49	1·29
25	25	0·81	0·59	1·40
75	0	0·67	0·53	1·20
75	25	0·57	0·60	1·17

Inhibitor 2 (R_F 0·45 to 0·65 in butanol/ammonia), unlike inhibitor 1, appears to be a true growth-inhibiting substance which reduces the elongation of coleoptile sections over a wide range of concentrations. It does this without causing damage to the tissue for, if such inhibited sections are subsequently transferred to IAA solutions, normal growth is resumed (Table II). Thimann and Bonner (18) and Leopold *et al.* (6) suggest that certain naturally occurring growth inhibitors such as protoanemonin, coumarin and chelidonic acid produce their effects by competing with auxin molecules for the -SH groups of protein cysteinyl units, and have shown that inhibitions due to these compounds can be relieved by the addition of -SH-rich substrates such as 2 : 3-dimercapto-1-propanol (BAL = British anti-Lewisite). Experiments carried out with *Malus* inhibitor 2 in combination with various concentrations of BAL showed no relief of inhibition either in the presence or absence of IAA, suggesting that the inhibition of growth caused by this natural inhibitor is of a different nature from that induced by coumarin or chelidonic acid. When applied to coleoptile sections whose growth was partially inhibited by a supra-optimal concentration of IAA (100 p.p.m.), *Malus* inhibitor 2 failed to reverse the inhibition; instead, the inhibitions due to the auxin and the inhibitor proved to be additive. This behaviour is again contrary to that reported for synthetic auxin-antagonists (6) which possess the property of reversing IAA-induced inhibition.

DISCUSSION.

The results presented in this paper concern only the auxins and growth inhibitors occurring in the acidic fraction of apple extracts. Substances with similar biological properties occur in the non-acidic fractions so that full discussion must await detailed examination of these non-acidic growth regulators. There are, nevertheless, several points of interest which call for comment at this stage.

Wright (20), working with the black currant, showed that the processes of growth and abscission in the fruit were controlled by different auxins, and the evidence for the apple, although less conclusive, points to the same conclusion. Previous papers in this series have described the correlation which exists between the periods of fruit drop in apples and the auxin content of the seeds (8, 11). The auxin studied in the earlier investigation was acidic in nature, was active in stimulating parthenocarpy in tomatoes, and was particularly abundant in the seeds during stage 2 of development. These observations make it reasonably certain that it may be identified with *Malus* auxin 3 in the present chromatographic analysis. By contrast *Malus* auxin 2, as already mentioned (p. 29), would appear to be associated principally with growth by cell extension and may therefore be the auxin that controls the growth rate of the fruit itself. The possible biological functions of auxin 1 and inhibitor 2 are at present obscure.

Of the acidic auxins found by Wright (20) one, on the basis of its R_F value, colour reactions and biological activity, was identified with reasonable certainty as IAA and the other was shown to be an unknown indole compound. None of the acidic auxins of the apple appears to be identical with any of those of the black currant. From these results and from those recently published by other workers (2, 15) it is becoming clear that we can no longer think in terms of growth control by a single auxin such as IAA, but that we have to deal with complexes of several auxins and inhibitors, each complex perhaps specific to particular genera or other groups of

plants. The apparent absence of IAA from the apple is of particular interest in view of the generally held belief that this compound is the principal naturally occurring auxin in plants.

SUMMARY.

1. The acidic fraction of ether extracts of mature apple leaves contains three substances which promote extension growth in sections of wheat coleoptiles. These are provisionally referred to as *Malus* auxins 1, 2 and 3. In addition there occur two substances which inhibit extension growth, referred to as *Malus* inhibitors 1 and 2. These substances have been separated by paper chromatography using a butanol/water/ammonia solvent. They may also be concentrated by adsorption on activated alumina from alcoholic solution.

2. Auxins 2 and 3 are active in stimulating root formation on hypocotyls of *Phaseolus vulgaris* and auxin 2, in addition, delays the abscission of delaminated *Coleus* petioles.

3. In the seeds during stage 1 of development the general pattern of auxins and inhibitors resembles that in the leaf. During the later stages of seed development auxin 3 and inhibitor 2 are particularly conspicuous on the chromatograms.

4. Dormant embryos contained only traces of free auxin. Following alkaline hydrolysis or after-ripening at 4° C., the production of auxin 2 could be demonstrated.

5. Auxin 2 was the dominant growth-promoting substance present in bursting fruit buds, in anthers and in the fruit flesh throughout the development of the fruit. This auxin was absent from the flesh of fruits infected with chat fruit virus.

6. None of the apple auxins could be identified as indoleacetic acid. They are also distinct from the auxins which occur in the fruit of the black currant.

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MOLYBDENUM AS A PLANT NUTRIENT

VIII THE EFFECTS OF DIFFERENT MOLYBDENUM LEVELS AND NITROGEN SUPPLIES ON THE NITROGEN FRACTIONS IN CAULIFLOWER PLANTS GROWN IN SAND CULTURE

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PREVIOUS papers in this series described the effects of different levels of molybdenum on the growth and on the ascorbic acid, chlorophyll, molybdenum, sugar, nitrate and total nitrogen contents of cauliflower plants grown in sand cultures with two levels of nitrate (1, 2, 3). A later paper (4) presented the results of similar investigations on the effects of different molybdenum levels given in the presence of different types of nitrogen supply. It was shown that the appearance of whiptail symptoms in molybdenum-deficient plants was independent of the form of nitrogen supply given. This paper presents results obtained for various nitrogen fractions, which, except for adenine, were determined in the same plants as those for which the results previously reported were obtained. Results of investigations on amino acid constituents by chromatography on filter paper are also given; they relate to plants grown in the experiments described previously and to plants grown on other occasions.

EXPERIMENTAL PROCEDURE.

Culture methods.

The sand culture methods used for growing the cauliflower plants (var. Majestic) and full details of the preparation of the molybdenum-free nutrient reagents required to supply nitrogen and other elements have been described previously (1, 4, 6). Nitrogen was given as nitrate at 12 mg. eq./l. in 1949 and at two levels of 6 and 24 mg. eq./l. in 1950. In 1951, 1952 and 1955 it was given as nitrate, nitrite or ammonium compounds at 10 or 12 mg. eq./l., or as urea or glutamic acid in equivalent amounts, sometimes in the presence of additional citrate or malate salts.

The molybdenum concentrations were 0·000005, 0·02 and 10 p.p.m. in 1949 and ranged in eight increments from 0·000005 to 19·2 p.p.m. in 1950. They ranged in four increments from 0·00001 to 50 p.p.m. in 1951 and in three increments from 0·000005 to 0·05 p.p.m. in 1952 (1, 4). The molybdenum levels were 0·00001 and 0·05 p.p.m. in 1955.

The experiments were designed as factorial layouts in two or six randomized blocks. In 1951 and 1952 the treatments were arranged so that plants given the same molybdenum supply were grouped in sub-blocks in which the nitrogen treatments were distributed at random, and the sub-blocks were also distributed at random in each

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replicate. In 1955 the six blocks were sub-divided to provide a split-plot arrangement in regard to the two molybdenum treatments.

Analytical methods.

Leaves of all ages were used, except those that were necrotic or senescent or under about 2 cm. in length. The following estimations were made in duplicate on each sample from the 1951 experiment: (*a*) total nitrogen in material dried at 80° C., ground in a Christy and Norris mill and digested by a semi-micro Kjeldahl method modified as described previously (3) to include nitrate; (*b*) nitrate in dry material, by the phenoldisulphonic acid method (3); (*c*) protein, as alcohol-insoluble nitrogen, on comparable sub-samples of fresh material preserved in 85 per cent. alcohol, macerated and finally washed with alcohol before Kjeldahl digestion; (*d*) total α amino nitrogen, by the semi-micro method of Van Slyke, MacFayden and Hamilton (14) on the alcohol-soluble fraction; (*e*) ammonia nitrogen in the alcohol-soluble fraction, by the Conway and O'Malley micro-diffusion method (5); (*f*) amide, from the increase in ammonia after hydrolysis of alcohol-soluble nitrogen fractions by 6N hydrochloric acid at 100° C. in sealed ampoules; (*g*) total organic nitrogen, as the difference between total nitrogen and the sum of nitrate and ammonia nitrogen, since hydroxylamine, nitrite and urea concentrations appeared to be negligible.

Adenine was determined by the method of Koritz and Skoog (11) which they developed for use with plant tissues. Samples weighing 10 gm. were used and duplicate estimations were made on aliquots equivalent to 0·4 gm. fresh weight. There was no appreciable interference by adenosine or 5′-adenylic acid and the analytical agreement was $\pm$ 0·93 μg. for values between 4 and 45 μg. Samples were stored at –18° C. for one or two days if necessary, during which time no loss of free adenine occurred. These estimations were carried out on the 6-block replicated factorial experiment in 1955.

The individual amino acids and amides were investigated, in plants grown between 1949 and 1952, by chromatographic separation on filter paper. The leaf samples were extracted with 70 per cent. alcohol. The extracts were concentrated *in vacuo* and applied to the starting line on the paper in amounts up to 20 μl. The single dimensional separations were descending chromatograms in phenol-ammonia or n-butanol-acetic acid-water (4 : 1 : 5) as solvents, while two dimensional separations were run first in the butanol solvent and then in phenol-ammonia. The chromatograms were dried at room temperature, sprayed with 0·1 per cent. ninhydrin in n-butanol and developed at 80° C. Estimates of the relative amounts of the individual compounds were made in terms of high, medium and low categories,or by comparison with standard spots prepared at the same time as the unknowns. The comparisons were made independently by two observers.

RESULTS.

Major nitrogen fractions.

The concentrations of total nitrogen, protein, α amino and other major nitrogen fractions, as found in 1951 in the leaves of plants aged 78 and 145 days, are shown for each treatment in Figs. 1 and 2 as percentages of fresh weight. The mean values of these fractions for different molybdenum levels and for different nitrogen treatments are given in Tables I and II respectively. Table III states the statistical significance

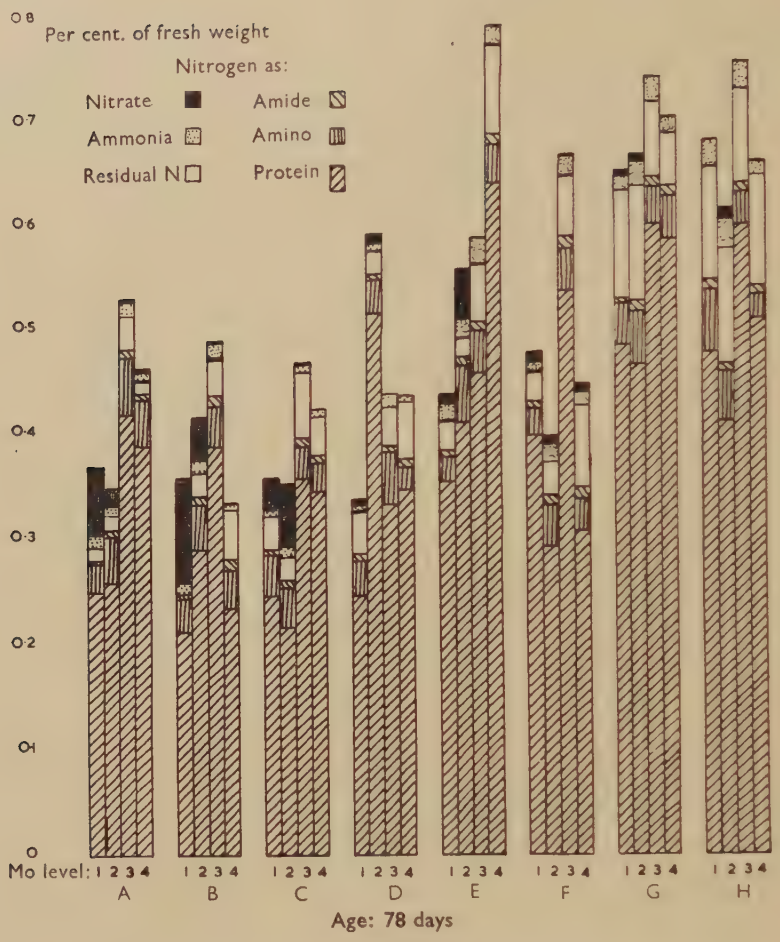

FIG. 1.

Nitrogen fractions as per cent. of fresh weight of leaves of cauliflower plants, aged 78 days, grown with different sources of nitrogen at the following molybdenum levels:

(1) 0·00001 p.p.m. (approx.)
(2) 0·00005 p.p.m.
(3) 0·5 p.p.m.
(4) 50 p.p.m.

For significance of overall molybdenum and nitrogen treatments see Table III.

Nitrogen Supply:

A Nitrate.
B Nitrate and carbonate.
C Nitrate and citrate.
D Nitrite.
E Ammonium nitrate.
F Ammonium sulphate.
G Ammonium citrate.
H Urea.

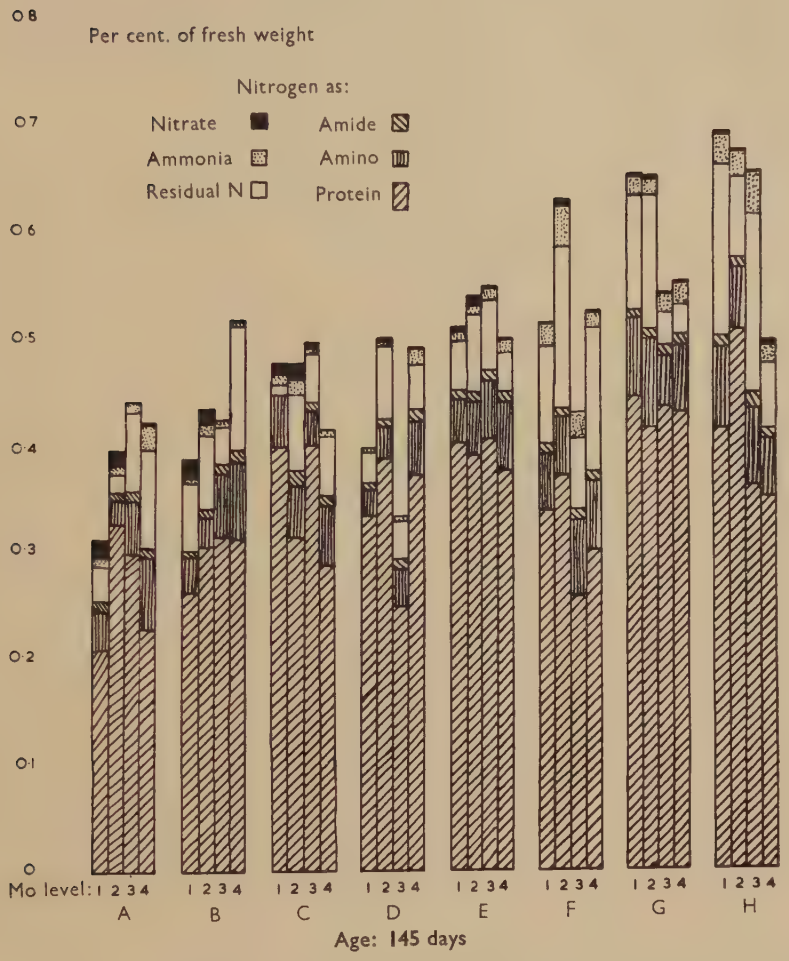

Fig. 2.

Nitrogen fractions as per cent. of fresh weight of leaves of cauliflower plants, aged 145 days, grown with different sources of nitrogen at the following molybdenum levels:

(1) 0·00001 p.p.m. (approx.)
(2) 0·00005 p.p.m.
(3) 0·5 p.p.m.
(4) 50 p.p.m.

For significance of overall molybdenum and nitrogen treatments see Table III.

Nitrogen Supply:

A Nitrate.
B Nitrate and carbonate.
C Nitrate and citrate.
D Nitrite.
E Ammonium nitrate.
F Ammonium sulphate.
G Ammonium citrate.
H Urea.

of the observed results, calculated both on a fresh weight and on a dry weight basis. The mean values are also given (Table II) for the effects of nitrogen treatments arranged in two groups: Group I, nitrate and nitrite; Group II, ammonium salts and urea. This is in order to reveal more clearly the distinction between them which provides the main contribution to the interaction between molybdenum and nitrogen.

TABLE I.

Effect of variation in molybdenum level on nitrogen fractions as per cent. of fresh weight in leaves of cauliflower plants; also mean values for all nitrogen treatments at each molybdenum level. 1951 experiment.

Fraction	Age of plants									
	78 days					145 days				
	Mo level (p.p.m.)				L.S.D. at 5% level	Mo level (p.p.m.)				L.S.D. at 5% level
	·000005	·00005	0·5	50		·000005	·00005	0·5	50	
Total N	0·461	0·495	0·587	0·537	0·092	0·497	0·542	0·487	0·494	0·050
Nitrate N	0·028	0·024	0·000	0·001	0·009	0·007	0·010	0·002	0·001	0·007
Ammonia N	0·012	0·015	0·018	0·010	0·006	0·012	0·015	0·014	0·014	0·006
Organic N	0·421	0·456	0·559	0·526	0·091	0·478	0·517	0·471	0·479	0·049
Amide N	0·006	0·007	0·008	0·009	0·0015	0·008	0·008	0·009	0·009	0·002
Amino N	0·036	0·043	0·039	0·032	0·009	0·049	0·047	0·053	0·062	0·008
Protein N	0·335	0·359	0·464	0·422	0·093	0·358	0·381	0·343	0·334	0·050
Residual N	0·044	0·047	0·058	0·063	—	0·063	0·081	0·066	0·074	—

Total nitrogen content was significantly greater in the ammonium salts and urea treatments than in the nitrate and nitrite treatments.

Nitrate concentration was significantly decreased by increased molybdenum supply. Nitrate accumulation was appreciable or significant only when nitrate was present in the nutrient and was greater in plants aged 78 days than in those aged 145 days, comprising up to 27 per cent. of the total nitrogen content of the former.

The ammonium nitrogen content was significantly affected by nitrogen supply at both periods. It was greatest in the plants given urea or ammonium compounds; plants given nitrate or nitrite had lower and similar ammonium concentrations. The mean concentration, for all nitrogen treatments, of ammonium nitrogen in 78-day plants was significantly greater with 0·5 p.p.m. than with 0·000005 or 50 p.p.m. Mo.

Total organic nitrogen showed, in plants of both ages, a significant effect of nitrogen source, concentrations being usually greater with the ammonium salts and urea than with the nitrate and nitrite treatments. The effects of changes in molybdenum level were less regular. At 78 days there was in general an increase up to 0·5 p.p.m. molybdenum and then a decrease to 50 p.p.m. The increase was more marked in the nitrate-nitrite than in the ammonium salt-urea group. The mean effect was significant but the interaction was not significant. At 145 days there was

Table II.

Effect of variation in nitrogen source on nitrogen fractions, as per cent. of fresh weight, in leaves of cauliflower plants; also mean values for all molybdenum levels with each nitrogen source. 1951 experiment.

Fraction	N source (Group I)					N source (Group II)					L.S.D. at 5% level for individual sources
	Nitrate	Nitrate + calc. carb.	Nitrate + citrate	Nitrite	Mean of Group I	Ammon. nitrate	Ammon. sulphate	Ammon. citrate	Urea	Mean of Group II	
Age 78 days											
Total N ..	0·428	0·402	0·405	0·452	0·422	0·596	0·500	0·694	0·683	0·620	0·130
Nitrate N ..	0·021	0·034	0·023	0·003	0·020	0·014	0·006	0·003	0·002	0·006	0·013
Ammonia N ..	0·008	0·009	0·008	0·009	0·009	0·018	0·014	0·021	0·023	0·019	0·009
Organic N ..	0·399	0·359	0·374	0·440	0·393	0·564	0·480	0·670	0·658	0·585	0·129
Amide N ..	0·006	0·008	0·006	0·007	0·007	0·008	0·009	0·008	0·008	0·008	0·002
Amino N ..	0·042	0·038	0·033	0·034	0·037	0·039	0·034	0·042	0·040	0·041	0·013
Protein N ..	0·329	0·282	0·293	0·362	0·317	0·468	0·386	0·539	0·502	0·474	0·131
Residual N ..	0·022	0·031	0·042	0·037	0·033	0·049	0·051	0·081	0·108	0·072	—
Age 145 days											
Total N ..	0·393	0·443	0·469	0·433	0·435	0·529	0·530	0·606	0·639	0·576	0·070
Nitrate N ..	0·008	0·008	0·008	0·001	0·006	0·004	0·008	0·001	0·002	0·004	0·009
Ammonia N ..	0·011	0·006	0·008	0·006	0·008	0·010	0·025	0·018	0·028	0·020	0·009
Organic N ..	0·374	0·429	0·453	0·426	0·421	0·515	0·497	0·587	0·609	0·552	0·069
Amide N ..	0·008	0·008	0·009	0·009	0·009	0·009	0·009	0·009	0·010	0·009	0·002
Amino N ..	0·043	0·047	0·046	0·035	0·048	0·053	0·061	0·069	0·066	0·062	0·012
Protein N ..	0·265	0·299	0·361	0·338	0·316	0·397	0·321	0·438	0·414	0·393	0·071
Residual N ..	0·058	0·075	0·037	0·044	0·055	0·056	0·106	0·079	0·119	0·090	—

again an increase with molybdenum level in the nitrate-nitrite group but a tendency to decrease in the ammonium salt-urea group. In consequence the main effect of molybdenum at this age was small and not significant, whereas the interaction was significant.

Amide concentrations were usually slightly, but significantly, increased by increase in molybdenum supply in plants aged 78 days.

Total α amino nitrogen concentrations at 78 days showed only small and non-significant changes with treatments. At 145 days there were highly significant mean effects of both molybdenum and nitrogen treatments and also interaction effects. The concentrations were greater in plants of the urea and ammonium salt group than in those of the nitrate and nitrite group. This distinction was particularly marked

TABLE III.

Significance of the observed mean effects of variation in molybdenum level and nitrogen source on nitrogen fractions in cauliflower plants aged 78 and 145 days; also of the observed interaction between molybdenum and nitrogen. Data calculated on fresh weight and on dry weight basis. 1951 experiment.

Fraction	Effect of Mo		Effect of N source		Interaction	
	78 days	145 days	78 days	145 days	78 days	145 days
Total N	N.S.	$\phi\phi$	XXX $\phi\phi\phi$	XXX $\phi\phi\phi$	N.S.	N.S.
Nitrate N	XXX $\phi\phi\phi$	X $\phi\phi$	XX $\phi\phi\phi$	$\phi\phi$	XXX $\phi\phi\phi$	N.S.
Ammonia N	X	N.S.	XX $\phi\phi$	XXX $\phi\phi\phi$	N.S.	N.S.
Amide N	XX	N.S.	N.S.	N.S.	N.S.	N.S.
Protein N	X	ϕ	XX $\phi\phi$	XXX $\phi\phi$	N.S.	N.S.
Amino N	ϕ	XX	N.S.	XXX $\phi\phi\phi$	N.S.	XX
Total organic N	XX	ϕ	XXX $\phi\phi\phi$	XXX $\phi\phi\phi$	N.S.	X

Significant at 5 per cent. level: X, on fresh weight basis; ϕ, on dry weight basis.
Significant at 1 per cent. level: XX, on fresh weight basis; $\phi\phi$, on dry weight basis.
Significant at 0·1 per cent. level: XXX, on fresh weight basis; $\phi\phi\phi$, on dry weight basis.
N.S., not significant.

at the low molybdenum levels. Increased molybdenum had little effect in the urea-ammonium salt group but markedly increased the α amino nitrogen concentrations in the nitrate-nitrite group. Even at normal or high molybdenum levels, however, concentrations in the latter were usually less than those at any molybdenum level in the urea-ammonium salt group.

Changes in protein content generally resembled those observed with total organic nitrogen; the effect of nitrogen source, namely a greater content in plants of the urea-ammonium salt group than in those of the nitrate-nitrite group, was significant at both ages. The effects of changes in molybdenum level were significant only at 78 days. There were usually increases in protein up to 0·5 p.p.m. molybdenum and these were more marked in the nitrate-nitrite group.

In view of the reported role of adenine as a leaf and bud growth factor (10), some estimates were obtained of its concentration in leaves of cauliflower plants aged 40-50 days. The concentrations of free adenine found, which are only about one-fifth of those reported for greenhouse-grown tobacco plants (11), are shown in Table IV.

Table IV.

Effects of molybdenum levels and nitrogen supply on mean free adenine content (μg./gm. fresh weight) of cauliflower foliage.

Molybdenum	N source		
	Nitrate	Nitrite	Ammon. sulphate
Present	36·0	55·0	80·0
Absent	17·5	65·0	75·0

L.S.D. for effect of Mo at 5 per cent. level, 15·0.
L.S.D. for effect of N source at 5 per cent. level, 12·5.
L.S.D. for effect of N source at 1 per cent. level, 17·5.
L.S.D. for effect of N source at 0·1 per cent. level, 22·5.

In the presence of molybdenum there was a marked effect of nitrogen source, the concentrations decreasing in the order ammonium sulphate, nitrite, nitrate, with each of the differences fully significant. In the absence of molybdenum the order of concentrations was the same and, while the difference between ammonium and nitrite sources was smaller and not significant, the decrease with nitrate was especially marked and highly significant. The effect of molybdenum was significant only in the presence of nitrate where it halved an already low concentration.

Distribution of total nitrogen between various fractions.

The relative percentages of the total nitrogen recovered in the different fractions estimated in 1951 are shown in Figs. 3 and 4.

The proportion of nitrogen present as nitrate decreased with increased molybdenum supply and also decreased in the interval between 78 and 145 days. The proportions of ammonium nitrogen were less in plants of the nitrate-nitrite than in those of the ammonium salt-urea group. In plants aged 78 days ammonium nitrogen concentrations tended to be least with 50 p.p.m. Mo. Amide nitrogen proportions were unaffected by nitrogen or molybdenum supplies and were fairly uniform.

There were usually lower proportions of α amino nitrogen at 78 days in plants of the ammonium salt-urea group than in those of the nitrate-nitrite group. The proportions of protein nitrogen at 145 days also were generally less in plants of the former group.

The proportions of residual organic nitrogen were markedly increased in the older plants and were often more than twice those found in the younger plants. The adenine nitrogen concentrations found in 1955, in plants similar to those grown in 1951, were equivalent to approximately 5-7 per cent. of the residual organic nitrogen figures obtained in these earlier experiments. Adenine nitrogen comprised 0·5-1·0 per cent. of the normal total nitrogen content of cauliflower foliage.

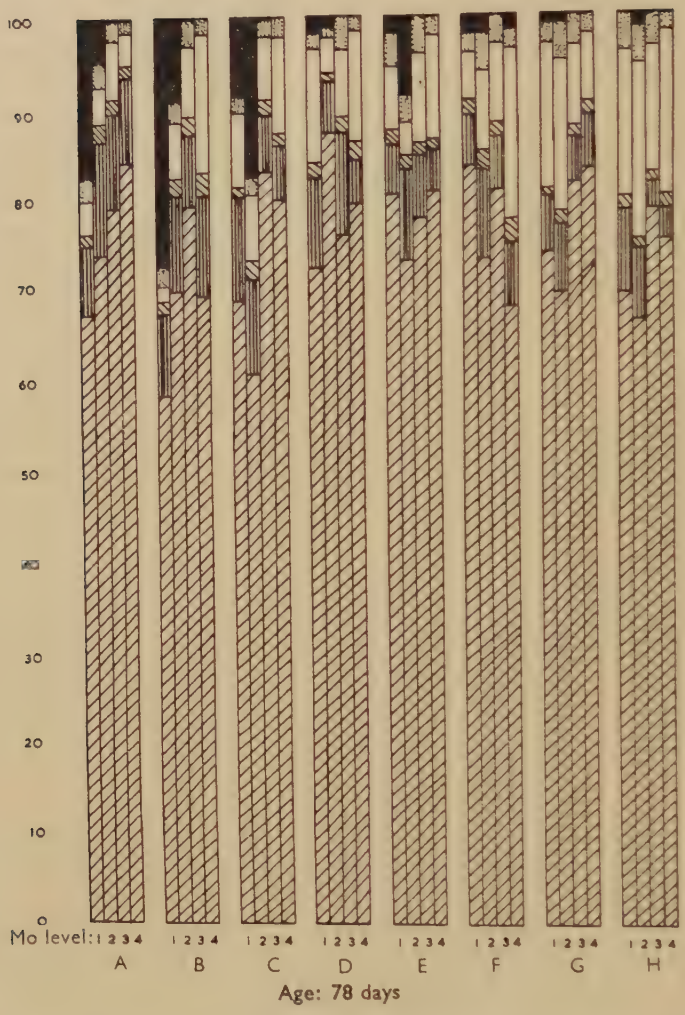

FIG. 3.

Distribution of nitrogen, as per cent. of total nitrogen content, between different nitrogen fractions (represented as in Figs. 1 and 2) in leaves of cauliflower plants, aged 78 days, grown with different sources of nitrogen at the following molybdenum levels:

(1) 0·00001 p.p.m. (approx.)
(2) 0·00005 p.p.m.
(3) 0·5 p.p.m.
(4) 50 p.p.m.

Nitrogen supply:

A Nitrate.
B Nitrate and carbonate.
C Nitrate and citrate.
D Nitrite.
E Ammonium nitrate.
F Ammonium sulphate.
G Ammonium citrate.
H Urea.

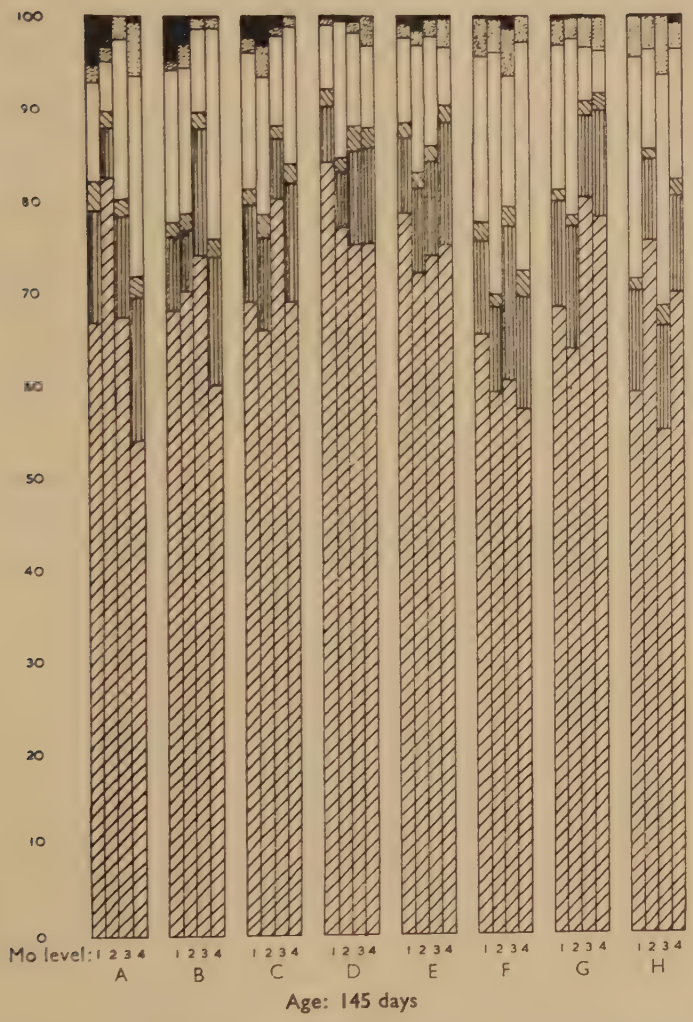

Fig. 4.

Distribution of nitrogen, as per cent. of total nitrogen content, between different nitrogen fractions (represented as in Figs. 1 and 2) in leaves of cauliflower plants, aged 145 days, grown with different sources of nitrogen at the following molybdenum levels:

(1) 0·00001 p.p.m. (approx.)
(2) 0·00005 p.p.m.
(3) 0·5 p.p.m.
(4) 50 p.p.m.

Nitrogen supply:

A Nitrate.
B Nitrate and carbonate.
C Nitrate and citrate.
D Nitrite.
E Ammonium nitrate.
F Ammonium sulphate.
G Ammonium citrate.
H Urea.

Chromatographic examination of amino acid compounds.

Plants were sampled at intervals to investigate the effects of molybdenum supply on their amino acid content. Preliminary results obtained in 1948 have been reported earlier (9).

1949 series. Cysteic acid, or a labile precursor, appeared only in extracts from molybdenum-deficient plants and was associated with mature rather than with young leaves. In parallel experiments cysteic acid was observed in swede, marrow-stem kale, Hungry Gap kale, brussels sprouts and rape grown under similar conditions of molybdenum deficiency and was consistently absent from normal plants.

Glutamic acid concentrations were consistently increased with increase in molybdenum supply over the range from "nil" to 10 p.p.m. Alanine, glutamine and arginine showed similar increases in concentration with increase in molybdenum supply from "nil" to 0·02 p.p.m. but seldom showed further increases with 10 p.p.m. Mo. Similar but smaller changes were observed for asparagine, valine, serine, threonine and the isomeric leucines, all of which were usually present at much lower concentrations than glutamic acid or alanine. Plants were affected severely by molybdenum deficiency in this series.

1950 series. Cysteic acid was detected in extracts of young leaves from plants given 0·000005 or 0·00005 p.p.m. Mo but not from plants given excess molybdenum (19·2 p.p.m.); in extracts of old leaves from these plants it was detected at all three levels of molybdenum supply. Glutamic acid concentrations increased with progressive increases in molybdenum supply. Glutamine concentrations appeared to decrease as the molybdenum supply increased. The plants were somewhat less severely affected by molybdenum deficiency, even at the lowest level, than in the 1949 series.

1951 series. Glutamic acid concentrations increased with increase in molybdenum supply from the deficiency levels of 0·000005 and 0·00005 p.p.m. Mo to the intermediate and high levels of 0·5 and 50 p.p.m. in all nitrogen treatments, except nitrate with added sodium citrate where the reverse was observed. Glutamine appeared to be the principal amino compound in plants given 0·5 p.p.m. Mo and was present in uniformly high concentrations regardless of nitrogen source. With increasing molybdenum supply, changes in glutamine concentrations and in the much smaller amounts of aspartic acid were similar to those of glutamic acid.

At the 0·5 p.p.m. level of molybdenum, arginine concentrations were much higher in plants given urea or ammonium compounds than in those given nitrate or nitrite. Arginine was greatly increased at molybdenum deficiency levels in plants given urea or ammonium compounds, including ammonium nitrate, and was probably the principal amino compound present in these plants. The concentrations of arginine were low in plants given nitrate only but showed changes with molybdenum level similar to those in plants given ammonium compounds. The severity of molybdenum deficiency in plants given nitrate at the lowest level was similar to that observed in 1950.

1952 series. Glutamic acid was consistently decreased by molybdenum deficiency, both in plants grown with nitrate as the only source of nitrogen and in those to which nitrate and nitrite, or nitrate and ammonia were given in equal amounts. In the other treatments, which provided nitrogen as nitrite, ammonium compounds, glutamic acid or urea, glutamic acid was markedly increased by molybdenum deficiency. The concentrations of glutamine, which were usually higher than those of

glutamic acid, showed similar trends and became especially high in molybdenum-deficient plants grown with urea, glutamic acid or ammonium compounds. Similar changes in response to molybdenum supplies occurred in the relatively small concentrations of aspartic acid.

The concentrations of arginine were highest in plants grown with urea, glutamic acid or ammonium compounds (including ammonium nitrate) and they attained especially high values in such plants when grown without molybdenum. Arginine concentrations were low and often below the limits of detection in plants grown with nitrate or nitrite only, but with nitrite the amounts were appreciably increased by molybdenum deficiency.

The concentrations of serine were increased by molybdenum deficiency in the presence of nitrite, urea, glutamic acid or ammonium compounds other than the nitrate, but were small and not consistently affected in plants given nitrate. The severity of molybdenum deficiency at the lowest level, as judged from the plants grown with nitrate treatments, was comparable to that observed in 1950.

DISCUSSION.

It was shown previously (4) in several experiments that molybdenum-deficient cauliflower plants developed symptoms of whiptail regardless of whether nitrogen was supplied as nitrate, nitrite, ammonium salts, urea or glutamic acid. The results, presented here, of one of these experiments show that such molybdenum-deficient plants, when supplied with nitrogen as ammonium salts or urea, contained, by comparison with normal plants given nitrate and molybdenum, adequate amounts of total α amino nitrogen, protein, total organic nitrogen and adenine. The molybdenum-deficient plants given nitrogen sources of the ammonium salt-urea group were thus provided with substantially greater amounts of these fractions than deficient plants in the nitrate-nitrite group. It is therefore concluded on these grounds that the occurrence of whiptail symptoms is not the result of the possible effects of inadequate amounts of these fractions in molybdenum-deficient plants.

In several molybdenum-deficient plants grown with nitrate as the source of nitrogen, there were marked decreases in the concentrations of some individual amino acids, including glutamic acid, aspartic acid and alanine. Such changes were to be expected from the specific role of molybdenum in the nitrate reductase system (12). On the other hand, in molybdenum-deficient plants grown with ammonium salts, urea or glutamic acid, there were often marked increases in the concentrations of certain amino acids or amides, and especially of arginine for which the most consistent and striking differences were observed. The effects of molybdenum deficiency on the amino acid pattern would appear, therefore, to depend on the type of nitrogen source used for growing the plants. It might be concluded tentatively that, in those nitrogen treatments where molybdenum deficiency does not limit organic nitrogen production, its effect is often to cause accumulation of certain amino acids. If this response to molybdenum deficiency is a general one, it might be expected that the concentrations of certain amino acids in molybdenum-deficient plants grown with nitrate would be determined by the net effect of two contrasting trends. Thus the effects of limited nitrate reduction in decreasing amino production as a whole would be partially offset by the tendency for certain amino acids, notably arginine, to accumulate provided sufficient organic nitrogen production could take place. This

view would offer some explanation for the frequent observation of inconsistent effects of molybdenum deficiency on the concentrations of arginine and some other amino acids in plants grown with nitrate, either alone or in combination with other nitrogen sources. Small changes in molybdenum supply during growth under deficiency conditions might result in one or other of the contrasting responses appearing as the dominant effect.

Earlier experiments with cauliflower grown with nitrate and several levels of molybdenum (1) suggested that the molybdenum requirement in nitrate reduction might be mainly satisfied at levels that were inadequate for some other requirement for normal growth. It may be relevant to the present discussion to recall that these and subsequent experiments (1, 4) showed that the production of whiptail in molybdenum-deficient plants grown with nitrate is associated with a somewhat critical molybdenum supply around 0·00005 p.p.m. This level is slightly greater than that producing the most severe depression on growth, together with "primary" symptoms of mottling, cupping and scorching of older leaves (4), whereas in plants provided with ammonium salts, urea, nitrite or glutamic acid, whiptail symptoms occur at the lowest molybdenum levels so far attainable, and indeed these levels are often obligatory for the production of such symptoms.

The possibility to be inferred from this discussion, namely that production of whiptail symptoms might be associated in some way with the increased concentration of a specific amino nitrogen compound that could occur either with a critical level of molybdenum in plants grown with nitrate, or at the lowest molybdenum levels in plants given other sources of nitrogen, is consistent with the observation by Steinberg (13) that external supplies of L(+) isoleucine and L alloisoleucine induce specific symptoms of leaf malformation known as "frenching" in tobacco in sterile cultures. Although the results of the present work do not permit any definite conclusions regarding the relationships between whiptail and the occurrence of unusually high concentrations of specific amino acids, the observations of Steinberg regarding isoleucine, and the effects of molybdenum deficiency on arginine reported here, suggest that such a relationship may occur. In the 1952 experiment (4) the effects of an external supply of arginine in the nutrient solution were tested in this connection but were entirely negative. It is possible, however, that continued investigations may reveal the existence of other compounds which, like arginine, increase under molybdenum deficiency but whose significance in respect of abnormal growth effects has not been appreciated up to the present.

SUMMARY.

(1) Cauliflower plants var. Majestic were grown in sand culture with nitrate, nitrite, ammonium compounds, urea or glutamic acid as sources of nitrogen at deficient, normal and high levels of molybdenum.

(2) Total nitrogen content increased with increase in molybdenum supply in plants grown with nitrate. Similar changes occurred in plants aged 78 days given other nitrogen sources but were not revealed in plants aged 145 days. Plants with nitrate usually contained much less total nitrogen than those grown with other sources of nitrogen.

(3) Nitrate content greatly increased in plants given nitrate with inadequate molybdenum supply. Other plants contained small or negligible amounts of nitrate.

Protein and total organic nitrogen were decreased by molybdenum deficiency in plants grown with nitrate.

(4) Molybdenum-deficient plants grown with ammonium compounds or urea usually contained as much protein, α amino nitrogen and total organic nitrogen as normal plants grown with nitrate and adequate molybdenum.

(5) Molybdenum deficiency resulted in decreases in the concentration of glutamic acid in plants grown with nitrate; under these conditions cysteic acid appeared, especially in mature leaves.

(6) Plants grown with ammonium compounds or urea contained relatively high concentrations of arginine. Molybdenum deficiency resulted in marked increases in concentrations of arginine and often of other amino acids in plants given these sources of nitrogen.

(7) Free adenine concentrations were greatest in plants given ammonium sulphate and were significantly less in those given nitrite or nitrate. Molybdenum deficiency decreased the adenine concentration only in plants given nitrate.

(8) It is concluded that whiptail symptoms of molybdenum deficiency in cauliflower are not caused by deficiencies of adenine, or of total α amino acid, organic or protein nitrogen fractions. The possibility that accumulation of a specific amino acid compound may be associated with the occurrence of whiptail is discussed, and merits further investigation.

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THE WYE COLLEGE PLANT GROWTH ROOMS

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THE value of a controlled environment for plant studies is widely recognized and there are now several types of installation operating in this country. In view of the difficulties experienced in obtaining information concerning the constructional details and performance of a relatively small unit, the data here presented may prove useful to other plant science laboratories in planning rooms to meet their requirements. The rooms described below were built without specialist labour except for the installation of the refrigeration units.

Three rooms were required for research purposes, in which the temperature could be simply controlled at any level for day and night over the normal range for plant growth, viz. 35°F.–90°F., and which would provide a light intensity adequate for normal development. To permit suitable replication in experimental treatments it was necessary to accommodate 60-80 tomato plants up to the 10-leaf stage and it was also necessary to place the rooms in an existing ground-floor laboratory. The rooms were planned to operate with 5-foot fluorescent tubes which appear to be the most efficient type of illumination for the purpose. With these considerations in mind the internal dimensions of each room were as follows: height 6 ft. 6 in., width 4 ft. 6 in., and length 8 ft. 3 in. The rooms are illustrated in Figs. 2 and 3.

Each unit is equipped with 20 fluorescent tubes (5 ft., 80 watts) of the "New Warm White" type which are mounted *outside* the rooms on a frame backed with sheet aluminium and placed immediately above a ceiling light of toughened plate glass ($\frac{5}{16}$ in. × 66 in. × 39 in.) (Fig. 4). A hand winch serves to lift the three frames of tubes simultaneously and thereby facilitate cleaning of the glass and replacements of the tubes. The light transmission figure for polished plate glass is 85-90 per cent., which is rather higher than that quoted for double-glazing. Chokes, condensers and compressor units are mounted on the roof of the room which they control.

The plants are supported beneath the light panel by a cable-slung wooden bench (5 ft. × 2 ft. 6 in.). A hand winch permits ready adjustment of the bench to any required height, not only to allow for accommodating plants of various sizes but also to facilitate control of light intensity at plant level.

For research purposes it is important to recognize that, with this simple arrangement of tubes, the distribution of light on the benching is uneven. In Fig. 1 the mean light intensities, measured along the bench with an Everett Edgcumbe Autophotometer, are shown for varying distances from the plate glass. A serious falling-off in light intensity is evident at the ends of the bench when it is close to the glass. The edge effect is similar, but less marked, across the narrow axis of the bench. At 12 in. from the glass, tomato plants in the centre of the bench are shorter and stockier than those at the two ends. This effect might be reduced to some extent by the installation of wall lighting or by the use of reflectors. Vince *et al.* (1956) have

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described an illumination outfit designed to minimize this defect, but this was not entirely suitable for the small units here described. For temperature and day-length studies with 70-80 plants, it has been found that setting the bench 2 ft. from the glass and changing the position of the plants every other day results in a very uniform batch of plants.

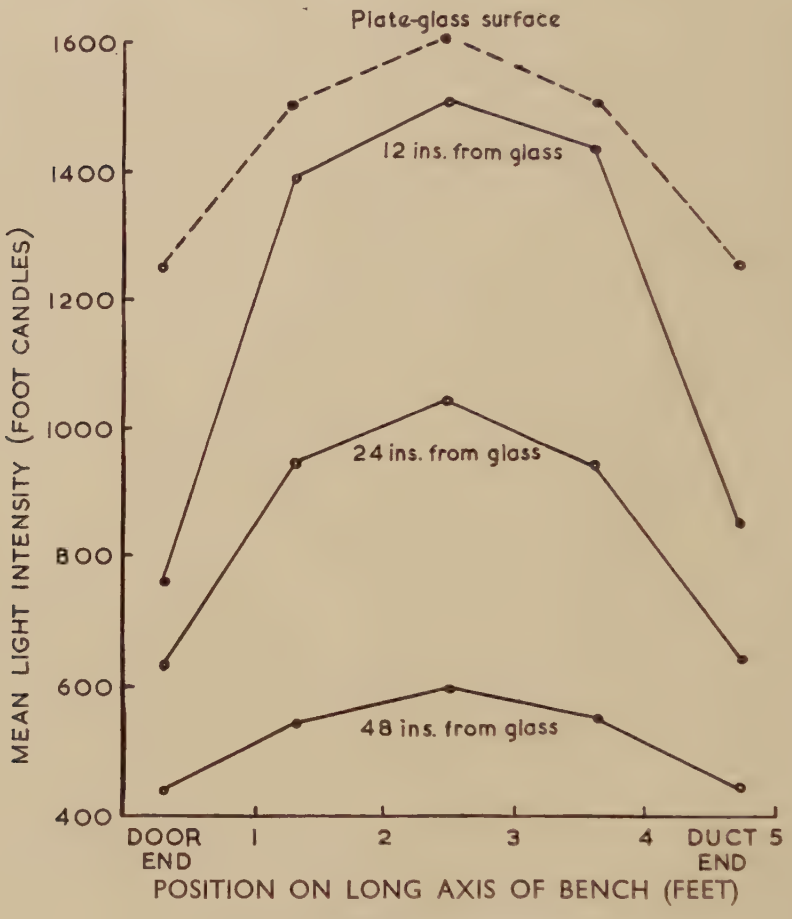

Fig. 1.
Light intensities in growth room.

Against the short wall opposite the door is an air duct constructed of 22 s.w.g. galvanized sheet steel, suitably stiffened with angle iron and extending from the ceiling to within 10 in. of the floor. Within this duct are located the heaters, cooling unit and control thermostats. Air from the room is forced across the cooling and heating units by means of a 12 in. fan operating continuously and located at the top of the duct. For ease of adjustment the four contact thermometers of the thermostat

PLATE I

Fig. 2.
Exterior view of plant growth rooms.

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FIG. 3.
Interior view of one room.

FIG. 4.
Illumination outfit.

system are fitted in a protective cover on the room side of the duct. Air from the duct is forced across each mercury bulb through ¾ in. diameter holes in the ducting, by small metal scoops which project into the air stream. The cooling unit is placed centrally in the duct and is operated by one ½ H.P. compressor. Immediately beneath the cooling unit are two one-kilowatt gilled heating units. Independent maximum and minimum safety thermostats (Teddington) are provided to prevent damage in the event of failure in the control relay system. Each unit has two time switches to permit the independent control of "day" length and of "day" and "night" temperatures.

With the lights on, temperature can be controlled at any level between 90° F. and 35° F. with an ambient temperature, in the room in which the chambers are installed, of between 60° F. and 70° F. The thermostats may be set to within 0·01° F., but in practice, to avoid continuous cutting in and out of the heating and cooling units, the thermostat in the heater circuit is set 1·5° F. below that for the cooling circuit. With the bench 24 in. from the glass, the temperature variation at points on the bench is from 0·5°-1·0° F. With the lights off and an ambient temperature of 65° F., a temperature of 27° F. was recorded and maintained after 3 hours. For the change-over from a "day" temperature of 70° F. to a "night" temperature of 50° F. equilibrium is reached in about 2 hours. The rise from 50° F. to 70° F., with lights and heaters operating, takes about 45 minutes.

The framework of the rooms is of 4 in.×2 in. timber covered with expanded metal. The cavity walls thus formed are filled with glass wool and the expanded metal is faced with plaster incorporating hair in the first coat and a suitable water repellent in the second. The floors are formed of a foamed-slag aggregate with the inclusion of a suitable waterproofing agent. It is necessary to provide a sturdy ceiling in view of the weight of equipment it has to support. The framework is of deal (4 in.×4 in.) and is plastered and lagged as for the walls. The outer sides of the ceiling are sealed with ¾ in. boards which make a strong base for fixing the electrical gear.

Many plants, including the tomato, grow well in a dry atmosphere if the roots are adequately supplied with water. Humidity control may thus be of little consequence for many types of investigations into effects of temperature or nutrition. By fitting an atomizer nozzle to the high-pressure water main in the room, the relative humidity was raised from 64 per cent. to 95 per cent. at 65° F. Without a humidifier the soil in three-inch pots tended to dry out rather rapidly when temperatures were high.

Very satisfactory results have been obtained by growing tomato plants in water culture, when no humidifier is necessary. The seed is germinated in silver sand and the plants transferred to 1 lb. jam jars as soon as the cotyledons appear. The solution is changed weekly and no further aeration is necessary. Using this technique, studies are in progress on the interaction of temperature and mineral nutrition on infection of tomato seedlings with viruses and root-infecting fungi.

For small-scale laboratory work this type of installation is invaluable since experiments can be repeated with reasonable accuracy, which is frequently impossible in an ordinary glasshouse where meteorological factors are so variable and difficult to control with precision. The total cost of constructing each room was approximately £500, of which £300 represented expenditure on electrical equipment and £200 the cost of building and other materials and labour.

SUMMARY.

The constructional details and performance are given for three growth rooms each designed to accommodate 60-80 plants in 3 in. pots. Illumination is by 20 "New Warm White" 80-watt fluorescent tubes arranged above a plate-glass ceiling panel. With the lights on, temperature control may be obtained at any level between 35° F. and 90° F. and different day and night temperatures may be provided, with a time lag of up to two hours for a drop of twenty degrees from day to night levels.

ACKNOWLEDGMENTS.

Sincere thanks are due to Messrs. W. Hollands, L. Lilley and L. Vane of the Maintenance Staff for their care and skill in constructing the rooms and the prototype from which these were finally designed.

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VIRUS DISEASES OF PEARS IN ENGLAND

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COMPARED with the many known in apples and stone fruits, few virus diseases of pears have been studied. Stony pit has been reported in several countries (Kienholz, 1939; Wallace, Ogilvie and Swarbrick, 1944; Atkinson, 1948; Mulder, 1951), and leaf symptoms have been termed mosaic (Christoff, 1935) and ring pattern (van Katwijk, 1954), but no investigations of possible indicator plants or attempts to develop virus-free clones have been described. Observations in orchards and nurseries in England suggested that virus diseases were prevalent in pear trees and their effect in stunting plants of some varieties seemed considerable. In the course of selecting healthy material on which the effect of virus infection could be assessed, possible indicator varieties and indexing methods have been investigated. This paper records the symptoms of the virus diseases and the technique now adopted for routine indexing.

SYMPTOMS ON NATURALLY INFECTED TREES.

In addition to stony pit, the virus symptoms observed on pear trees (and related species) in England fall into the following groups:

1. *Mosaic:* chlorotic patterns, usually associated with the veins but not typically in the form of vein banding (Fig. 1); the pattern is usually greenish-yellow, not the cream colour of apple mosaic. The intensity of the symptoms differs with variety, Beurré Hardy and Doyenné d'Eté being the most expressive of the varieties infected here.

2. *Vein yellows:* a narrow yellow banding of the veins, particularly of the tertiary and smaller ones (Fig. 2). This disease has been seen frequently on many varieties of pears, most commonly in nurseries because the symptoms develop more clearly in young than in mature trees.

3. *Red mottle:* dark red or bronze vein banding and flecking, developing on mature pear leaves from July onwards and most conspicuously in September before autumn coloration has occurred (Fig. 3).

4. *Quince vein banding:* a broad, light green vein banding has been observed on quince trees. In one tree of the clone Quince C, commonly used as a rootstock for pears, this symptom (Fig. 4) was associated with a virus infection distinct from that causing pear mosaic. The Quince C virus, which will be referred to as the "quince stunt virus", does not usually cause leaf symptoms either in Quince C or in pears.

5. *Hawthorn ring pattern:* a ring and vein banding pattern (Fig. 5) on a naturally infected hawthorn (*Crataegus oxyacantha*) was caused by a virus transmissible to quince, but symptoms in this host differed from those produced by the mosaic and quince stunt viruses.

TRANSMISSION TESTS.

Quince indicators.

In experiments begun in 1950, 8 one-year-old seedlings of Quince E (Portugal Quince) and 3 one-year-old layers of each of the clonal quince rootstocks A, B and C

(Hatton, 1920) were bud-inoculated with scions from one of three sources: (1) pear mosaic in an old tree of unknown variety; (2) vein yellows in a nursery tree of Doyenné du Comice; and (3) quince vein banding in a 5-year-old tree of Quince C. Hawthorn ring pattern virus was inoculated into Quince B plants.

The test plants were observed for 3 years and no symptoms were seen on the Quince B or C plants, except for those budded with hawthorn, and they developed a ring and line pattern. The Quince A plants inoculated with the virus from Quince C were symptomless, but those inoculated with pear mosaic and with vein yellows developed ring pattern and vein clearing respectively in the second year after inoculation.

The Quince E seedlings, however, developed symptoms in the year after inoculation and, despite variation between the plants, the symptoms in each inoculation group were distinct. The pear mosaic virus induced a yellow ring and line pattern (Fig. 6). The vein yellows inoculation induced a clearing and necrosis of the veins (Fig. 7), symptoms which later proved to have been caused by a virus latent in the pear source, and not by the virus causing pear vein yellows. The quince vein banding inoculum caused chlorotic spots and a marked distortion and crinkling of the leaves. In subsequent years, the chlorotic spots did not develop and the leaf symptoms resembled the ring and line pattern caused by pear mosaic; unlike those inoculated with mosaic virus, however, the plants inoculated with virus from Quince C were severely stunted (Fig. 8).

Pear and quince indicators.

In 1951, six virus sources were used to inoculate Quince E seedlings, pear seedlings and one-year-old trees of Beurré Hardy on Quince C rootstocks. Six Beurré Hardy trees, six quince seedlings and three pear seedlings were budded with chips from each source. The records of symptoms developing on the test plants during 3 years after inoculation are summarized in Table I.

This experiment clearly established the superiority of Quince E seedlings over Beurré Hardy and pear seedlings as indicators of all these pear viruses except red mottle.

TABLE I.

Comparison of pear and quince reactions to viruses from six sources.

Virus source	Leaf symptoms on			
	Source	B. Hardy	Pear seedlings	Quince E seedlings
Quince C	None	None	None	Chlorotic spots, crinkle, stunt
Bristol Cross ..	None	None	None	Sooty ring spot
Doyenné d'Eté ..	Mosaic Red mottle	Ring pattern Red mottle	Ring pattern Red mottle	Chlorotic spots, ring and line-pattern
Fondante d'Automne	Red mottle	Red mottle	Red mottle	Sooty ring spot
Early Market ..	Red mottle	Red mottle	Red mottle	Sooty ring spot
Apple mosaic .. (virulent strain)	Mosaic	—	Yellow vein banding	Large chlorotic areas on distorted leaves

The symptoms induced by apple mosaic virus on pear and quince seedlings were distinct from those caused by the viruses from pear (Fig. 9).

The occurrence of a new symptom type, called sooty ring spot, on quince seedlings inoculated with two sources showing red mottle suggested that both symptoms might be caused by the same virus. The absence of red mottle from the Bristol Cross source, which also induced sooty ring spot on Quince E, indicated the presence of two different viruses, however, and later tests have supported this conclusion.

The diagnostic feature of sooty ring spot is the development of black pigment in the epidermis of the leaf, giving the superficial appearance of a sooty mould; the pigment is frequently in the form of rings around the margins of pale yellow spots (Fig. 10) and is accompanied by vein yellowing and necrosis.

Tests on Crataegus, Malus and Sorbus.

Young seedlings of apple, hawthorn and mountain ash (*Sorbus aucuparia*) were chip-budded with quince seedlings that had been infected for two years with viruses causing mosaic, sooty ring spot, stunt, and hawthorn ring pattern. With the exception of hawthorn seedlings inoculated with ring pattern, none of these test plants developed clear symptoms. It was concluded that suitable indicator plants for pear viruses were unlikely to be found in these species and that none of the viruses concerned was in the apple mosaic group.

New quince indicators.

Seedlings of Quince G and of Meech's Prolific quince were less uniform and more prone to a yellow variegation (genetical) than those of Quince E; but although the latter had proved to be the most reliable indicators tested, they were unsatisfactory because they were too variable in symptom expression and too susceptible to mildew. Even when infected as 3-month-old seedlings in May, maintained in the glasshouse and cut back twice during the summer, they showed no symptoms until the following spring. The quince clones used as rootstocks for pears were not virus-sensitive, and new clones selected for their value as indicators and resistant to mildew were required. Although the selection programme, started in 1953, is still incomplete, two clones, C7/1 and C7/2, propagated from selected Quince E seedlings, have shown promise in extensive tests on quince rootstocks.

For these tests, the rooted shoots were harvested separately from each stool to be indexed. Four stocks from each stool were planted in the nursery row, the remainder being bedded for future tests or for propagation, and two were budded with the quince clone C7/1 and two with C7/2 in 1954. Ten stools of each of the rootstock clones Quince A, B, C, F, G, Raymond VII and SA65, six of each of Quince D and E, and 45 of a collection of 24 Provence clones (Tydeman, 1948), were all treated in this way.

In the following spring, the stocks were cut back to within six inches of the scion bud and stock shoots were removed according to routine nursery practice.

Both quince indicator clones behaved alike but their behaviour varied according to the variety of the rootstock (Fig. 11). Of the 40 buds inserted in Quince A stocks, only one failed to "take" and only one died later; the remainder grew into symptomless plants. Of the 40 buds on Quince B, only one did not "take", but the rest grew very slowly and, after making a few inches of weak growth, all the scions died by mid-July. On Quince C, all the buds remained dormant and had died by mid-May. The indicators on the other clones in the Malling series behaved as on Quince A. The

Provence clones varied: on some the indicator scions grew normally, on others they died as on Quince B, and on two clones they grew but developed the ring pattern characteristic of infection with pear mosaic virus.

This test was repeated in 1955, using the bedded stocks from the same stools of Quince B and C. Buds of Quince A and Quince E were also inserted on the stocks for comparison with those of C7/1 and C7/2. In the spring of 1956, C7/1 and C7/2 buds behaved as in the previous test, and those of Quince E similarly, but the scion buds of Quince A grew normally.

From the results of other tests (to be described later in this paper), it is concluded that the Quince B clone is infected with sooty ring spot virus and Quince C with stunt. It seems more probable that the two types of reaction shown by the buds of C7/1 and C7/2, viz. bud-death and dieback after delayed growth, were caused by the stunt and the sooty ring spot viruses respectively, rather than by some more obscure form of "incompatibility". Incompatibility between quince stock and quince scion has not been reported and that between quince and pear is not of this necrotic type. Both indicator clones behaved alike on each sub-clone being tested and, while their behaviour was uniform on each of the Malling clones, on the Provence quinces it varied with the individual sub-clone (stool) under test, i.e. plants in the stoolbeds of each Provence type were not uniformly infected as were those of the Quince B and C clones.

Vein yellows.

Vein yellows was observed on nursery pear trees propagated from mother trees without symptoms, and other observations on the distribution of this symptom also suggested that it might be due to growth conditions, not to virus infection. A transmission test with Williams' Bon Chrétien was started in 1954, when 10 Quince A stocks were budded with Williams' clone 1, on which vein yellows had been recorded, 10 with Williams' clone 2, on which no symptoms had been seen in the two previous years, and 10 with buds of both clones. In the following spring the buds of clone 1 on the double-budded stocks were rubbed off and those of clone 2 allowed to grow out. By July the symptoms of vein yellows were equally conspicuous on the clone 1 trees and on all ten clone 2 trees inoculated with clone 1 buds, but no symptoms developed on the uninoculated clone 2 trees.

Association of vein yellows and sooty ring spot.

Like the Doyenné du Comice in the first transmission test described above, most of the pear sources that transmitted sooty ring spot to the quince indicators in the 1950-1954 tests were themselves showing vein yellows. Furthermore, many of the quince indicators developed vein clearing before the sooty rings appeared. It seemed possible, therefore, that vein yellows in pear and sooty ring spot in quince were caused by one virus. The few apparent anomalies might be attributed to poor symptom expression either by the pear source or by the quince indicators (seedlings).

Tests on material collected in 1954 by Dr. A. Beryl Beakbane from apparently healthy pear trees in France, however, showed that the two symptom types are probably caused by distinct viruses. The collection consisted partly of material from seven trees of Williams', seven of Beurré Hardy, six of Clapp's Favourite, and seven of Jeanne d'Arc, four varieties that develop clear symptoms of vein yellows. These 27 sub-clones were propagated by budding each on to five Quince A rootstocks and two pear seedlings, and they were each virus-tested by inoculating three Quince E

seedlings. From the incidence of vein yellows on the pear clones and of sooty ring spot on the quince indicators shown in Table II, it seems unlikely that they were caused by the same virus. It is evident also that few of the pear trees represented in the collection from France were free from both viruses.

Stony Pit.

Kienholz (1939) reported that veinlet chlorosis was "probably" caused by the stony pit virus on certain varieties of pear. The vein yellows prevalent on pears in England was not found to be associated with fruit symptoms. The trees listed as virus sources in Tables I, II and III were producing normal fruit; furthermore, one tree of Catharine pear with severely distorted fruit was itself without leaf symptoms and Quince E seedlings, inoculated with buds from it in two successive years, developed no symptoms.

Although it cannot be concluded that the stony pit virus does not produce leaf symptoms on pear until trees inoculated with different sources have fruited for several years, it seems improbable that the symptom types described in this paper were caused by the stony pit virus.

Table II.

Incidence of vein yellows and sooty ring spot infection in pears.

Variety	Sub-clone No.	Symptoms on pear clone	Symptoms on quince indicators
Williams' Bon Chrétien	336	Vein yellows	Sooty ring spot
	337	Vein yellows	Sooty ring spot
	338	Vein yellows	None
	339	Vein yellows	None
	340	None	None
	341	Vein yellows	Sooty ring spot
	342	None	None
Beurré Hardy	343	Slight vein yellows	Sooty ring spot
	344	None	Stunt
	345	None	None
	346	Vein yellows + Red mottle	None
	347	Slight vein yellows	Sooty ring spot
	348	Vein yellows	None
	349	Vein yellows	None
Clapp's Favourite	350	None	Sooty ring spot
	351	None	None
	352	Slight vein yellows	None
	353	Vein yellows	Sooty ring spot
	354	Vein yellows	Sooty ring spot
	355	None	None
Jeanne d'Arc	356	Vein yellows	None
	357	Vein yellows	Sooty ring spot
	358	Vein yellows	None
	359	Vein yellows	None
	360	Vein yellows	Sooty ring spot
	361	Vein yellows	Sooty ring spot
	362	Vein yellows	None

DISTRIBUTION OF INFECTION IN COMMERCIAL VARIETIES.

Between 1952 and 1955, 115 trees representing 20 different pear varieties and 22 quince plants from rootstock stoolbeds were tested for virus infection by inoculating quince seedlings. Most of these trees either were already being used as sources of propagation material or were potential sources of healthy scion wood. The significance of red mottle as a virus symptom was not appreciated until after the trees had been indexed; it was then impossible to inspect them all as some had been destroyed, so that the incidence of red mottle given in Table III is probably below that actually occurring. The distribution of vein yellows has been omitted from Table III because of uncertainty in diagnosis on orchard trees but, as mentioned before in this paper, its incidence is probably similar to that of sooty ring spot.

As shown in Table III, some of the source trees proved to be infected with more than one virus, most of the complex infections being a combination of red mottle and sooty ring spot. In complexes involving the stunt virus, however, other virus symptoms may be masked by the severe effect of the stunt virus on the indicators.

Among the quince plants indexed were ten from the stool beds of Quince A and eleven of Quince C. Judged by the reaction of the quince seedling indicators, none of the Quince A was infected, but all of the Quince C plants were carrying the stunt virus and this had killed most of the infected test plants by the end of the second year after inoculation.

TABLE III.

Distribution of infection with four viruses among 23 varieties of pear and quince.

Variety tested	Number trees tested	Number of trees infected with				"Healthy"†
		Stunt	Sooty ring spot	Mosaic	Red mottle *	
Beurré d'Amanlis ..	1		1			
Beurré Superfin ..	4	1			1	2
Bristol Cross	2		1		1	1
Clapp's Favourite ..	2		1		1	
Conference	7		4		2	2
Doyenné d'Eté	4	1	1	1	4	
Doyenné du Comice ..	7	1	4		4	1
Dr. Jules Guyot ..	1					1
Early Market	1	1	1			
Emile d'Heyst	1	1	1			
Fondante d'Automne ..	3		1		3	
Glou Morceau	3	1			3	
Gorham	2					2
Beurré Hardy	25	2	7	4	7	5
Josephine de Malines ..	2	1	1		1	
Laxton's Superb ..	7	1	4		4	2
Packham's Triumph ..	1	1				
Unknown	1		1			
Williams' Bon Chrétien ..	18		5		4	13
Winter Nelis	1	1				
Quince A	10					10
Quince B	1		1			
Quince C	11	11				
Totals	115	23	34	5	35	39

* Symptoms observed on the pear source.
† Many of these were infected with vein yellows.

PLATE I

Fig. 1.
Mosaic in Beurré Hardy.

Fig. 2.
Vein yellows in Doyenné du Comice.

Fig. 3.
Red mottle in Beurré Hardy.

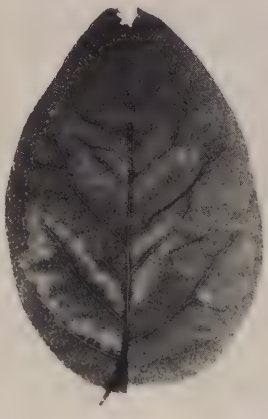

Fig. 4.
Vein banding in Quince C.

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FIG. 5.
Ring pattern in hawthorn.

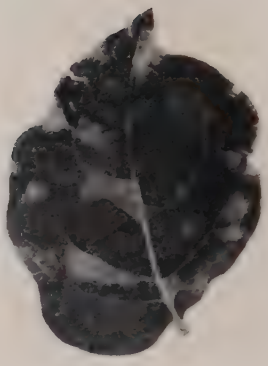

FIG. 9.
Apple mosaic in Quince E seedling.

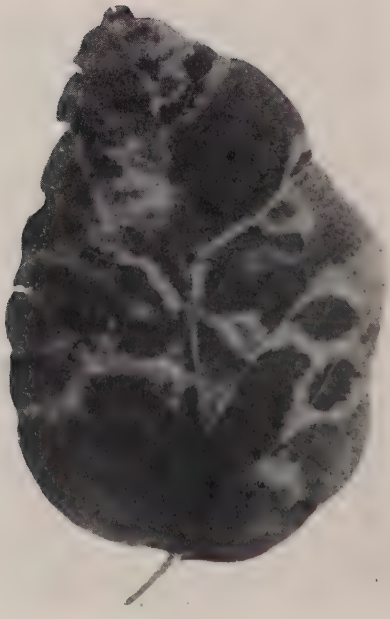

FIG. 6.
Pear mosaic in Quince E seedling.

FIG. 7.
Vein clearing in Quince E seedling inoculated with sooty ring spot virus.

FIG. 8.

Effect of the stunt virus from Quince C on Quince E seedlings; two on left inoculated in 1952, uninoculated controls on right; photographed in September 1953.

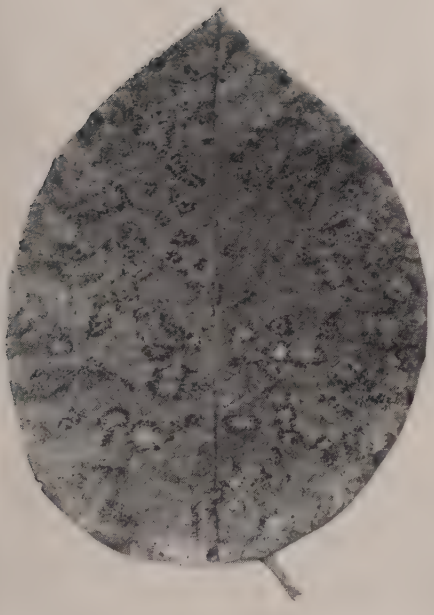

FIG. 10.

Sooty ring spot symptoms in Quince E seedling.

FIG. 11.

Reaction of indicator clone C7/1 on (l. to r.) Quince A, B and C.

The one plant of ungrafted Quince B that has been indexed on quince seedlings was carrying sooty ring spot virus. The prevalence of this virus in pears grafted on Quince B rootstocks, and the uniform reaction of the C7 indicators budded on plants from the ten stools of Quince B, suggest a high incidence of infection in this clone.

DISCUSSION.

The only virus disease of pears that has caused concern among growers is stony pit, from which the damaged fruit is obvious both in the orchard and in the packing shed. Diseases that cause a reduction in crop, either by restricting tree size or by lowering fertility, attract the grower's attention only when there are conspicuous leaf symptoms, or when healthy and diseased trees of the same variety are growing in the same orchard.

The investigation reported in this paper and observations over a period of five years have shown that virus diseases other than stony pit are prevalent in the pear clones in commercial use. Usually the material of a variety being propagated in any particular nursery is uniformly infected, so that neither the nurseryman, nor the fruit-grower with an orchard planted with trees from one nursery, has the opportunity of comparing healthy and infected trees.

There is no evidence that these diseases are of recent origin; indeed, the distribution of mosaic in old orchards suggests that this has occurred in pears for at least 50 years. The tolerance of the quince rootstock clones, compared with the sensitivity of most quince seedlings, may be the outcome of unintentional selection for tolerance to virus diseases in the past. Sensitive clones would have been discarded as unsatisfactory rootstocks. It seems probable, however, that two nursery practices have contributed to the spread of virus infection in recent years.

The use of the Quince C clone as a dwarfing rootstock will have spread the quince stunt virus not only to pears grown on this stock, but also to others propagated from scions of trees on Quince C.

The second practice contributing to virus spread from one variety to another is "double-working" (Garner and Hammond, 1940) to avoid the weak graft-union characteristic of certain scion varieties on quince rootstocks. An intermediate scion of a compatible variety is interposed between stock and incompatible variety, and any virus infecting this intermediate scion is transferred to the top scion. The transmission of mosaic from Beurré Hardy and of vein yellows and red mottle from Conference to other varieties are examples of such transmission that have been observed in nurseries. The problem of whether or not latent virus infection influences the strength of the graft union is now being studied, but clearly material that has passed the most stringent tests for virus infection is a prerequisite of such an investigation.

Apart from transmission from stock or intermediate to scion, and perpetuation of infection in scionwood, no means of spread of the viruses that infect pear are yet known; but if spread by insect vectors does occur in orchards, its importance would be insignificant compared with the high incidence of infection already introduced from the nursery. There is evidence that vectors do not spread these viruses in nurseries; in our experiments no uninoculated seedlings have shown symptoms over two and three-year periods of exposure to insects, although growing adjacent to plants experimentally and naturally infected with the viruses mentioned above. To instance

specific diseases, quince stunt has not spread from Quince C stoolbeds to adjacent Quince A stoolbeds during some 35 years at East Malling. A clone of Williams' Bon Chrétien has remained entirely free from vein yellows though it has been propagated among infected nursery material of the same and other varieties for at least 5 years.

The spread of these diseases in orchards, if it occurs, and the losses they may be causing the fruitgrower cannot be estimated until the general position has changed from one in which most pear trees are infected to one in which a substantial proportion are healthy. If the difference that can be seen between healthy and infected trees in the nursery is reflected in the later performance of the trees, the overall loss of fruit to the growers would be of the order of 15 or 20 per cent., an annual cess to which no industry would wish to submit indefinitely.

The immediate precaution to be taken is the use of healthy rootstocks and scion material as soon as they can be made available. As the Quince A clone, already the most widely used rootstock for pears in Britain, is apparently virus-free in the East Malling stoolbeds, the provision of healthy rootstocks presents no difficulty. The selection of healthy scion material of commercial pear varieties, on the basis of indexing tests, is now in progress and the products will be distributed through the Mother Tree scheme of the East Malling Research Station (Posnette and Hoblyn, 1956).

SUMMARY.

An investigation of the virus diseases occurring in commercial stocks of pears and in quince rootstocks has shown a high incidence of infection with viruses causing five types of symptoms. Three types, mosaic, vein yellows and red mottle occur on pear; two types, stunt and sooty ring spot, develop on quince and the viruses are latent in pear.

Of the species and varieties tested, Quince E seedlings proved to be the most reliable indicators; clones derived from two of these reacted characteristically when budded on to quince rootstocks infected with either mosaic, sooty ring spot or the stunt virus.

The evidence from observations and tests on a range of rootstocks and scion varieties suggests that the high incidence of infection is due primarily to the use of infected material in propagation. The selection of virus-tested material of commercial varieties for distribution is in progress.

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BOOK REVIEWS

ORNAMENTAL TREES. AN ILLUSTRATED GUIDE TO THEIR SELECTION AND CARE. By Evelyn Maino and Frances Howard. (California: University of California Press; London: Cambridge University Press. 1956. Pp. 219, illus. Price 28s.)

This book is described by the authors as "an attempt to anticipate and answer the questions which would occur to the home owner". The modesty of this claim conceals the fact that it is an invaluable reference work for anyone interested in amenity plantings, whether home owner, landscape architect or town planner.

The great merit of the book—and in this it scores where so many others fail—is the clarity of the layout on its 10 × 8 in. pages, coupled with the streamlined style in which the information is presented. It contributes little to the science of arboriculture but it effectively fills the gap in the literature between the encyclopaedia and the-chapter-on-ornamentals-in-any-gardening-book.

An eleven-page introduction deals succinctly with the use and care of trees. The main part of the book is divided into four sections: deciduous trees, 58 genera, 105 species; broad-leaved evergreen trees, 41 genera, 71 species; conifers, 20 genera, 51 species; palms and other tropicals, 10 genera, 15 species. There are full descriptions and cultural notes for 182 of these trees, one per page, under the headings: feature, use, conformation, flowers, fruit, root system, rate of growth, natural requirements (climate and soil), care (pruning, feeding, watering, pests and diseases) and faults (such as short lived, litter-producing, brittleness of branches). The wide margins have Latin and English names and synonyms—the correct Latin pronunciation is included—and line drawings of foliage, flowers and/or fruit, and an outline shape of the mature tree. Charts at the end of each section summarize the important items, the information included varying with the group concerned, e.g. for deciduous trees autumn colour and winter habit are two of the thirteen columns, whereas for conifers the chart shows climatic tolerance only.

Being an American publication, the book includes species suitable for sub-tropical climates. But even with these omitted, it retains its value to gardeners in Britain. The text is free of mistakes, the selection of species is sufficient for the most fastidious and the information will satisfy the most exacting enquirer. In short, an excellent addition to the range of horticultural books. G.A.B.

DICTIONARY OF GARDENING. SUPPLEMENT. Editor: P. M. Synge. (Oxford: Clarendon Press. 1956. Pp. 334, illus. Price 42s.)

The Royal Horticultural Society have now issued the First Supplement to their invaluable Dictionary of Gardening. This is divided into two parts, the first containing lists of recommended garden varieties of flowers, fruits and vegetables, each in its own section, the second recording additions and corrections to the main work with various articles on subjects omitted previously or, by reason of recent progress, requiring fresh treatment.

Garden hybrids, by recent decree of the International Committee of Horticulture, are henceforth to be known by the unlovely name of "cultivar", the term "variety" being now reserved for varietal forms occurring in the wild. Truly the language of

science becomes daily more barbarous. The more earthy of the gardening brotherhood will find this uncouth Esperantoish combination difficult to assimilate. It is doubtful if they will try. The decision came too late for the changes to be included in this volume, a reprieve for which the Editors show a discreetly veiled gratitude.

The lists of recommended "cultivars" (to try it on the dog) are selections of the best and most reliable varieties in the opinion of those who have studied or specialized in these particular plants. It does not follow that the reader and the specialist will always agree, though differences of opinion are more likely to concern omissions than inclusions. The authors having nothing to sell, these lists provide reliable descriptions untinged with commercial enthusiasm and thus can be accepted without the usual grain of salt. Some are comprehensive to a degree, others less so. The rose list describes 500 varieties, which should be enough. The viola list of 45 names seems less adequate, but possibly only because it omits several of the present writer's favourites. The fruit section provides comprehensive descriptive lists of all the main varieties (e.g. 156 apples, 72 pears, and so on), each followed by a short list of the varieties recommended as combining the four most necessary characters, those of productivity, hardiness, suitable growth and fruit quality. A reprint is included of Crane and Brown's fertility rules for planting. The vegetable lists read like a seedsman's catalogue, but one from which all chaff has been winnowed. The potato lists give the "form" of each variety and note any idiosyncrasies which might influence choice.

In Part 2 the additions and corrections call for no special comment. The special articles, however, will prove valuable, in particular those on organic fungicides (inorganic fungicides were dealt with in the main volume), gas and fog injury, electrical heating of frames, and some of the newer insecticides, to mention a few. There are also 13 columns setting out the International Code of Nomenclature for Cultivated Plants as devised by the 13th International Horticultural Congress in 1952 or by a committee thereof, in a gallant and ingenious attempt to standardize the naming of garden hybrids. The complications are such that, like the man in that other Floral Dance, we could only stand and stare, but legal minds should love it.

This supplement fully upholds the high standard set by the main volume, that is to say that it is a worthy adjunct to the most authoritative and complete book on English gardening so far produced.

G.St.C.F.

PLANT PROPAGATION PRACTICES. By James S. Wells. (New York and London: The Macmillan Company. 1956. Pp. 344, illus. Price $7.50 or 42s.)

Members of the *Plant Propagators' Society* of America and readers of the *American Nurseryman*, who have long enjoyed and profited by Mr. Wells' articles on nursery procedure in relation to the latest findings of experimenters, will be delighted to have this comprehensive work. Others may perhaps be forgiven if they look twice at the price but they can be assured that the author gives full value. Born and educated in England, Mr. Wells went to the United States to be successively propagator to three very large nurseries where up-to-date techniques were well tested on a large commercial scale, thus obtaining a thorough, and possibly unique, experience. The acid test for research findings is to try them against the background of the cheque book. This has been done and the author now gives us his considered views clearly

and concisely. Like the many factors leading to success in propagation, which must be taken whole, so with this book we are not to dip, but to go "straight through the first time".

The author first tells us how to set up a propagating unit, selecting the site in relation both to natural features and to trading facilities, conserving and improving the soil—even to the extent of contour planting and ley farming—whilst making full use of the official advisory services. Next are considered propagating structures, including deep frames for frost protection, removable cross walls to facilitate cultivation by machine and the installation of humidification apparatus. Sash-built houses are less expensive than permanent structures and lath houses and portable lath screens are essential; full descriptions and working drawings are provided. The efficient control of water, heat and light leads to success and to learn how best to use them is the major problem for the propagator. Principles having been dealt with clearly and concisely, and always in relation to practice, procedures next receive attention and this is where we "start propagating". Whilst the principles have general application, the procedures are confined almost entirely to woody plants, the shrubs and trees of our gardens. Seeds come first, as usual, but probably only to clear the air for cuttings, upon which the nurserymen chiefly depend. Details are given of heat regimes, hormone treatments, wounding, rooting media and the use of humidification and constant mist under glass and in the open.

The author has tried mist culture on a large scale and finds it good. A table of results, almost entirely for evergreens, gives rooting successes mostly above 80 per cent. These rooted cuttings are then potted and housed to establish and overwinter. The careful nursing of cuttings rooted under mist is clearly essential. Normal methods of grafting are described but the main value of this section lies in the numerous practical hints in both text and table. Layering, including the popular air-layering techniques, and the maintenance of stock by offshoots or division are discussed. The production of a well-balanced sturdy "liner" for field planting is given a chapter to itself and there are some fine pictures of mechanical planters at work. The principal plants of economic importance, including rhododendron, holly, magnolia and various conifers, are treated in greater detail in Part IV and there is a month-by-month programme starting in June, the beginning of the shrub propagator's year. An uninhibited appendix, referenced in the text, gives addresses of suppliers of everything a propagator desires, from advice, personal or written, to seeds, chemicals and gadgets. There is also a good index and a short list of recommended books.

This is an excellent work, clearly written by a propagator who has successfully married science to practice. R.J.G.

PUBLICATION RECEIVED.

THE NATIONAL INSTITUTE OF AGRICULTURAL BOTANY. THIRTY-SIXTH REPORT AND ACCOUNTS, 1955. 1956. Pp. 54.

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1. The **Journal of Horticultural Science** is an official organ of the Horticultural and Agricultural Research Station, Long Ashton, Bristol, and the Horticultural Research Station, East Malling, Kent.

The Publication Committee welcome horticultural papers from all other sources.

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5. Where a paper is of a highly technical nature it should be preceded by a brief **synopsis**, in which its contents are simply and clearly stated for the benefit of non-technical readers.

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